

**BIOSYSTEMATICS OF STACHYS  
SWAINSONII BENTH. (LAMIACEAE)  
and its relations to some other  
chasmophytic Stachys species**

**DAGMAR PERSSON**

lund 1981





BIOSYSTEMATICS OF STACHYS SWAINSONII BENTH. (LAMIACEAE) AND ITS  
RELATIONS TO SOME OTHER CHASMOPHYTIC STACHYS SPECIES

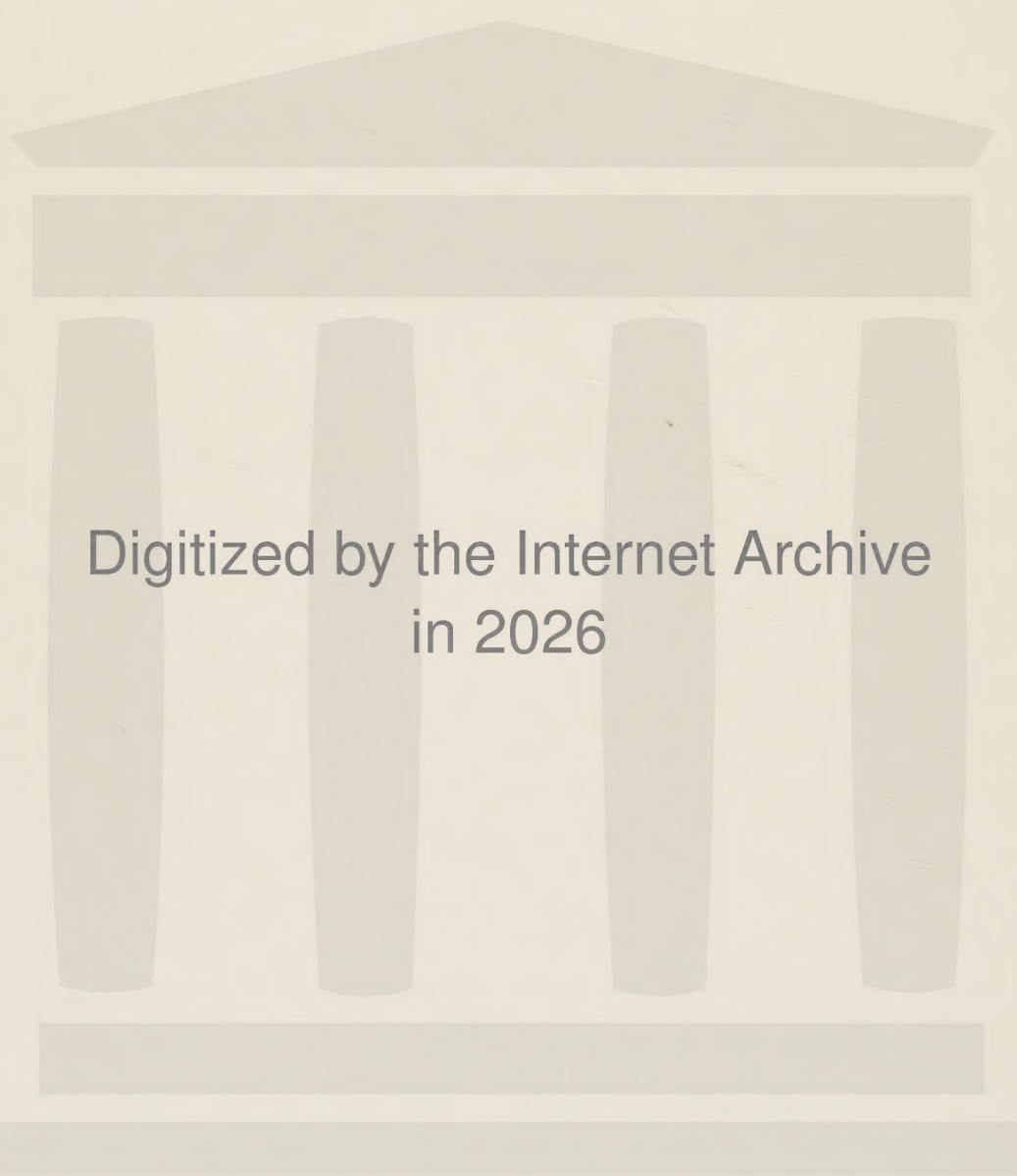
DAGMAR PERSSON

B.A., GB.

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| Abstract<br>The <i>Stachys swainsonii</i> group, endemic to C Greece, has been taxonomically revised. The following taxa are recognized: <i>S. swainsonii</i> ssp. <i>swainsonii</i> , ssp. <i>scyronica</i> , ssp. <i>melangavica</i> ssp. nov., ssp. <i>argolica</i> , <i>S. spruneri</i> and <i>S. ionica</i> . For comparison some more distantly related species, all endemic to S and C Greece, have been included: <i>S. candida</i> , <i>S. chrysantha</i> , <i>S. spreitzenhoferi</i> ssp. <i>spreitzenhoferi</i> , ssp. <i>virella</i> ssp. nov., <i>S. euboica</i> , <i>S. messeniaca</i> , <i>S. mollissima</i> and <i>S. tetragona</i> . A key, descriptions, nomenclature, illustrations and distribution maps are provided.<br>The chromosome number for all taxa is $2n=34$ .<br>All taxa are suffruticose chasmophytes confined to calcareous substrates.<br>Microhabitat-correlated variation was observed within populations of most taxa; morphologically and apparently also genetically differentiated variants (subpopulations) occupied different microhabitats. Interpopulation variation of morphological characters was comprehensively analysed in 15 populations of <i>S. swainsonii</i> and <i>S. spruneri</i> .<br>Comprehensive hybridization experiments were performed using all 14 taxa. Triple crosses were made to investigate the possible origin of <i>S. swainsonii</i> ssp. <i>melangavica</i> . The results show that the taxa are separated by incompatibility barriers of varying strength and suggest a reticulate pattern of relationships.<br>All taxa are presumably relicts of formerly more widespread species complexes. |  |                                        |                  |
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PERSSON, D. 1981. BIOSYSTEMATICS OF STACHYS SWAINSONII BENTH. (LAMIACEAE) AND ITS RELATIONS TO SOME OTHER CHASMOPHYTIC STACHYS SPECIES

The *Stachys swainsonii* group, endemic to C Greece, has been taxonomically revised. The following taxa are recognized: *S. swainsonii* Benth. ssp. *swainsonii*, *S. swainsonii* Benth. ssp. *scyronica* (Boiss.) Phitos et Damboldt, *S. swainsonii* Benth. ssp. *melangavica* D. Perss. ssp. nov., *S. swainsonii* Benth. ssp. *argolica* (Boiss.) Phitos et Damboldt, *S. spruneri* Boiss., *S. ionica* Hal. For comparison some more distantly related species, all endemic to S and C Greece, have been included: *S. candida* Bory et Chaub., *S. chrysantha* Boiss. et Heldr., *S. spreitzenhoferi* Heldr., *S. spreitzenhoferi* ssp. *spreitzenhoferi* Heldr., *S. spreitzenhoferi* ssp. *virella* D. Perss. ssp. nov. *S. euboica* Rech. f., *S. messeniaca* Boiss., *S. mollissima* Willd., *S. tetragona* Boiss. et Heldr. A key, descriptions, nomenclature, illustrations and distribution maps are provided.

The chromosome number for all taxa is  $2n=34$ .

All taxa are suffruticose chasmophytes. They are confined to calcareous substrates and no obvious differences in ecological demands have been observed. The taxa are allopatric with rare exceptions. Owing to their special habitat requirements and to the topography of the area most populations within taxa are spatially isolated and gene exchange cannot reasonably be expected.

Microhabitat-correlated variation has been observed in most taxa. Intra-population variation was experimentally investigated in the Delphi population of *S. swainsonii* ssp. *swainsonii*. The population could be divided into morphologically and apparently also genetically differentiated subpopulations adapted to different microhabitats. Interpopulation variation of morphological characters was comprehensively analysed in 15 populations of *S. swainsonii* and *S. spruneri*. Five allopatric population groups were recognized: *S. swainsonii* ssp. *swainsonii*, ssp. *scyronica*, ssp. *melangavica*, ssp. *argolica*

and *S. sprunerii*. *S. swainsonii* ssp. *argolica* was exceptionally variable at both intra- and interpopulation levels, the variation apparently involving characters found in *S. sprunerii* and *S. swainsonii* ssp. *swainsonii*.

Comprehensive hybridization experiments were performed using all 14 taxa. Triple crosses were made to investigate the possible origin of *S. swainsonii* ssp. *melangavica* -- *S. swainsonii* ssp. *melangavica* displays characters found in the other subspecies of *S. swainsonii* and in *S. sprunerii*. Whenever possible  $F_2$ ,  $F_3$  and  $F_4$  were included. Most taxa were readily crossed. Seed set in  $F_1$  and subsequent generations was low. Germination, which was generally variable, was fairly low in  $F_1$  and decreased in later generations. Pollen stainability was reduced in most hybrids and often varied widely within a combination. Chromosome pairing in meiosis was mainly good and abnormalities usually few. The results of the crossing experiments show that the taxa are separated by incompatibility barriers of varying strength and suggest a reticulate pattern of relationships. The subspecies of *S. swainsonii* are closely related, *S. sprunerii* is close to *S. swainsonii*, while *S. ionica* is more remote. The two subspecies of *S. spreitzenhoferi* are closely related. In spite of morphological similarities there is evidently genetic disharmony between *S. candida* and *S. chrysantha*. The other species used in the crossing experiments are more isolated.

All taxa are presumably relicts of formerly more widespread species complexes. During the paleogeographic history of the distribution area, climatic fluctuations were probably accompanied by fluctuations in population size which can have given rise to the present-day pattern of variation.



## CONTENTS

|                                                                               |    |
|-------------------------------------------------------------------------------|----|
| ACKNOWLEDGEMENTS                                                              | 5  |
| INTRODUCTION                                                                  | 7  |
| HISTORICAL OUTLINE                                                            | 9  |
| MORPHOLOGICAL NOTES                                                           | 15 |
| TAXONOMY                                                                      | 21 |
| Key to the species                                                            | 21 |
| <i>S. swainsonii</i> Benth.                                                   | 23 |
| <i>S. swainsonii</i> Benth. ssp. <i>swainsonii</i>                            | 25 |
| <i>S. swainsonii</i> Benth. ssp. <i>scyronica</i> (Boiss.) Phitos et Damboldt | 28 |
| <i>S. swainsonii</i> Benth. ssp. <i>melangavica</i> D. Perss. ssp. nov.       | 29 |
| <i>S. swainsonii</i> Benth. ssp. <i>argolica</i> (Boiss.) Phitos et Damboldt  | 30 |
| <i>S. spruneri</i> Boiss.                                                     | 31 |
| <i>S. ionica</i> Hal.                                                         | 33 |
| <i>S. candida</i> Bory et Chaub.                                              | 34 |
| <i>S. chrysantha</i> Boiss. et Heldr.                                         | 36 |
| <i>S. spreitzenhoferi</i> Heldr.                                              | 37 |
| <i>S. spreitzenhoferi</i> ssp. <i>spreitzenhoferi</i> Heldr.                  | 38 |
| <i>S. spreitzenhoferi</i> ssp. <i>virella</i> ssp. nov. D. Perss.             | 38 |
| <i>S. euboica</i> Rech. f.                                                    | 39 |
| <i>S. messeniaca</i> Boiss.                                                   | 40 |
| <i>S. mollissima</i> Willd.                                                   | 41 |
| <i>S. tetragona</i> Boiss. et Heldr.                                          | 43 |
| MORPHOLOGICAL VARIATION                                                       | 44 |
| Field observations                                                            | 44 |
| Greenhouse experiments                                                        | 47 |
| (a) Varied environment experiments                                            | 47 |
| (b) Transplant and progeny examinations                                       | 60 |
| (c) Cross-pollinations                                                        | 61 |
| (d) Conclusions                                                               | 62 |
| Statistical analyses                                                          | 63 |
| Material and methods                                                          | 63 |
| Analysis of variance                                                          | 67 |
| Coefficient of variation                                                      | 83 |
| Additional characters examined in the randomly collected material             | 84 |
| Discussion                                                                    | 91 |
| Conclusions                                                                   | 97 |



|                                                      |     |
|------------------------------------------------------|-----|
| CYTOLOGY                                             | 99  |
| Material and methods                                 | 99  |
| Chromosome number and morphology                     | 100 |
| Meiosis                                              | 102 |
| Chromosome number in the hybrids                     | 103 |
| CROSSING EXPERIMENTS                                 | 104 |
| Crossing programme                                   | 104 |
| Material and methods                                 | 105 |
| Fertility in parental taxa                           | 107 |
| Fertility tests in the hybrids                       | 111 |
| Seed set after cross-pollination                     | 111 |
| Germination of hybrid seeds                          | 113 |
| Hybrid inviability                                   | 113 |
| Morphology of $F_1$ hybrids                          | 115 |
| Pollen stainability in $F_1$ hybrids                 | 116 |
| Reciprocal differences in pollen stainability        | 122 |
| Seed set in $F_1$ hybrids                            | 126 |
| Germination of $F_1$ seeds                           | 129 |
| Morphology of $F_2$ hybrids                          | 129 |
| Abnormalities in the hybrids                         | 130 |
| Pollen stainability in $F_2$ hybrids                 | 132 |
| Seed set in $F_2$ hybrids                            | 134 |
| Later generations, $F_3$ and $F_4$ hybrids           | 136 |
| Backcrosses                                          | 139 |
| Tetragona crosses                                    | 139 |
| Triple crosses                                       | 139 |
| Comments                                             | 144 |
| Meiosis in the hybrids                               | 145 |
| Conclusions                                          | 151 |
| GENERAL CONCLUSIONS                                  | 153 |
| PALEOGEOGRAPHY OF THE AREA                           | 154 |
| REFERENCES                                           | 158 |
| APPENDIX 1. Populations used in crossing experiments | 163 |
| APPENDIX 2. Crosses                                  | 166 |

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## INTRODUCTION

The genus *Stachys* is one of the largest in Lamiaceae. Several of the groups within *Stachys* are known for their polymorphism, among them the *S. swainsonii* complex.

The taxa comprising this group are chasmophytes restricted to calcareous habitats and are endemic to central Greece. The extensive range of morphological variation, particularly in populations on the Argolis Peninsula and in the Delphi area, has caused taxonomic difficulties.

A comprehensive investigation was necessary to determine the factors underlying the wide variation and to estimate its taxonomic and evolutionary significance. The *Stachys swainsonii* group and some related species were therefore studied biosystematically from 1973 to 1978. This paper reports the results (a) of extensive field work throughout the distribution area including analysis of the variation by statistical methods, (b) of comparative cultivation and crossing experiments at the Botanical Garden, Lund, Sweden, (c) of cytological studies and (d) of examination of herbarium material.

In addition to my own collections from two field trips, in 1973 and 1976, material from the following herbaria has been studied (abbreviations according to Lanjouw and Stafleu 1964): B, G, K, LD, M, P, W and the Goulimi-Goulandris Herbarium at the Goulandris Botanical Museum, Kifissa, Greece.

R. von Bothmer, H. Runemark, S. Snogerup (Lund) and A. Strid (Copenhagen) have contributed with collections.

Descriptions are based on material collected in nature.

Lawrence (1951), Stearn (1973) and the Systematics Association Committee for descriptive Biological Terminology (in Taxon, 1962) were consulted for morphological terms and Kornerup and Wanscher (1974) for colours.

Geographical names are spelt as in the Times Atlas (1956) as far as possible. In the historical outline, however, the spelling used by the respective authors was preferred.

Populations used in crossing experiments are listed in Appendix 1.

A list of herbarium specimens examined is kept at the Botanical Museum in Lund.

For abbreviations of names of taxa see end of Historical outline.

## HISTORICAL OUTLINE

*Stachys swainsonii* comprises many local populations in the Delphi area, on the Isthmus of Corinth and on the Argolis Peninsula. In earlier accounts populations in the Ionian Islands were also included in *S. swainsonii* but they are now recognized as a separate species. The closely related *S. spruneri* is distributed mainly in Attiki (also Voiotia). The following summary of important points from the relevant literature reflects the confusion of taxa in the past. (Names of taxa and most localities are spelt according to the respective author.) Fig. 1 shows localities.

*Stachys Swainsoni* was described by Bentham (1834) from Mt Parnassus based on material collected by Swainson. Ithaca, one of the Ionian Islands, was also mentioned as a locality (comm. Webb) in the original description. A drawing of material from Ithaca collected by Parolini and Webb 1819, appeared in *Mem. Ist. Veneto* (1843); Visiani pointed out here that his plant differed in some characters when compared with Bentham's description of *S. swainsonii*.

Boissier described three new species with affinity to *S. Swainsoni*. In 1844 he recognized *S. Scyronica* from the Isthmus of Corinth (the Scyronides Rock). It differed mainly in its small flowers and small leaves and silky greyish indumentum.

The description of *S. Spruneri* Boiss. appeared in *De Candolle's Prodrumus* (1848) and was based on collections from monte Parnes Atticae. *S. Spruneri* was distinguished by hispid hairs and narrow elliptic leaves.

*S. Argolica* Boiss. from Naupliam Argolidis was also described in *De Candolle* (1848). Boissier (1853) pointed out that *S. Argolica* was about



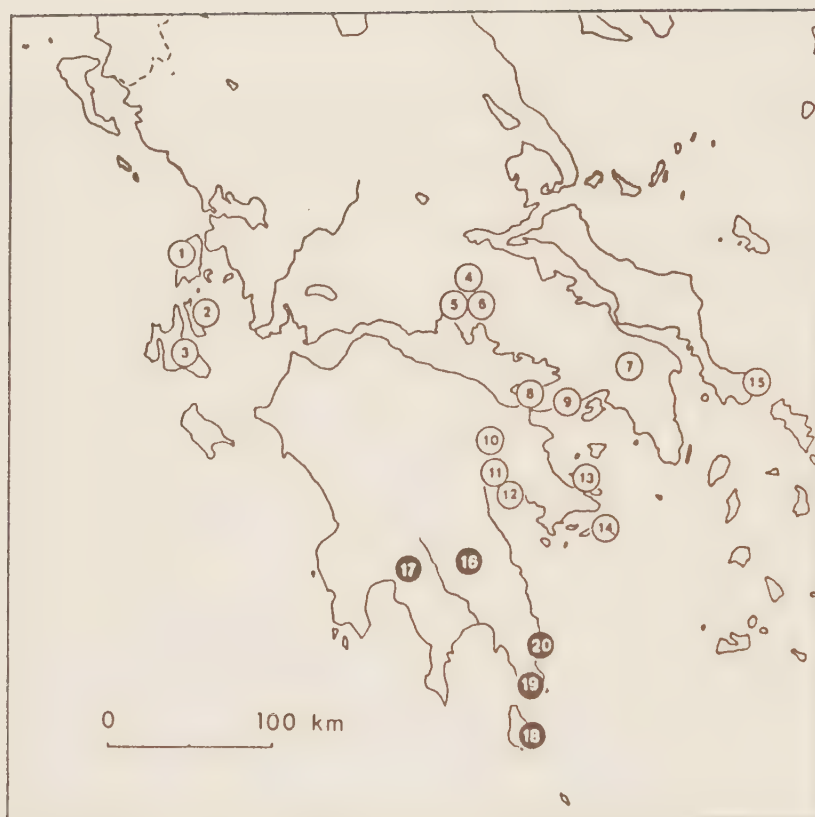


Fig. 1. Localities mentioned in the historical outline. The swainsonii group and *S. euboica* are shown in black numbers, the candida group in white.

|                 |              |                |                 |
|-----------------|--------------|----------------|-----------------|
| 1. Leukas       | 6. Rachova   | 11. Naupliam   | 16. Agriani     |
| 2. Ithaca       | 7. Mt Parnes | 12. Port Tolon | 17. Mt Taygetos |
| 3. Cephalonia   | 8. Lutraki   | 13. Methana    | 18. Cytherae    |
| 4. Mt Parnassus | 9. Megara    | 14. Hydra      | 19. Neapolis    |
| 5. Delphi       | 10. Mykenas  | 15. Euboea     | 20. Monemvasia  |

intermediate between *S. Spruneri*, from which it was somewhat more distant, and *S. Swainsoni* to which it was closer.

In Boissier (1853) *S. Scyronica* had been changed to a variety of *S. Swainsoni*.

In his flora Orientalis Boissier (1879) listed *Scyronica* as a variety, *S. Swainsoni*  $\beta$ . *Scyronica* and added Lutraki, Cephalenia and Ithaca to the locality list. *S. Argolica* was also changed to a variety of *S. Swainsoni* (*S. Swainsoni*  $\gamma$ . *Argolica*). Again, Boissier mentioned the relationship between *S. Spruneri* and var. *Argolica*, the former being distinguished by its narrow rigid leaves and broad floral leaves with an acuminate tip.

Heldreich (1883) in his Flore de l'île de Céphalonie recorded *S. Swainsonii* Benth.  $\beta$ . *Scironica* Boiss. from several localities on Cephalonia and Ostermeyer (1887) found it on Sta. Maura (Leukas).

From his travels in Greece in 1885 Haussknecht (1897) reported *S. Swainsonii* Bth. from Methana (Argolis) and var. *Argolica* Boiss. from Naupliam, Port Tolon and Mykenas.

Halácsy (1902) re-established var. *argolica* Boiss. as a species and listed var.  $\beta$ . *scyronica* as a synonym of *S. Swainsoni*. The distribution of his *S. Swainsoni* thus included Megara and Lutraki on the Isthmus of Corinth, Mt Parnassus and the Ionian Islands Cephalonia, Leucas and Ithaca.

Six years later Halácsy (1908) changed the rank of *scyronica* and recognized *S. Swainsoni*  $\alpha$ . *typica* and  $\beta$ . *scyronica*.

He also described a new species, *S. ionica* from the Ionian Islands, related to *S. Swainsoni* but differing in its silky indumentum, leaves with rounded base and long petioles, and calyx without glands. The variety  $\beta$ . *cephalonica* was distinguished by its glandular calyx.

Bornmüller (1928) accepted *S. Swainsonii* Benth. and f. *Argolica* Boiss. from Delphi. He interpreted some individuals of the Delphi population as *S. Argolica* Boiss. and remarked that he did not share Halácsy's opinion that *S. Argolica* Boiss. was a separate species. These plants with less dense

hispid pubescence and broadly oblong leaves with relatively long petioles -- in accordance with the diagnosis for *S. Argolica* -- grew on the same cliff face as the type for *S. Swainsonii* and should be considered only as a shade form of this species. Bornmüller also mentioned a herbarium specimen (Rachova, leg. Guicciardi; Herb. Graec. norm. no. 90) which Heldreich had already pointed out as an intermediate form of *S. Argolica* Boiss. He concluded that since they grew together in nature, the *argolica* form and the *typus* could not be separated in two species and besides *S. scyronica* should be regarded as a variety only.

Hayek (1929) kept *S. Swainsoni* and *S. argolica* as separate species. Under *Swainsoni* he recognized the forma  $\beta$ . *scyronica* Boiss. and the variety *B. ionica* (Hal.) (non-glandular) with a glandular form --  $\beta$ . *cephalonica* (Hal.).

Flora Aegaea (Rechinger 1943) deals entirely with the Aegean Islands and *S. argolica* is therefore the only species mentioned (from Hydra). None of the other taxa in the *swainsonii* group has been found on any of the Aegean islands.

In 1961 Rechinger described a new species related to *S. swainsonii*, *S. euboica*, from SE Euboea. The densely glandular plant with obovate or elliptic leaves is easily distinguished.

Phitos and Dambolt (1969) revised the *swainsonii* group. *S. ionica* Hal. was again re-established as a species since it differed from the other taxa in some constant morphological characters and was distributed only in the Ionian Islands. Three subspecies of *S. swainsonii* were recognized: ssp. *swainsonii*, ssp. *scyronica* (Boiss.) Phitos et Damboldt and ssp. *argolica* (Boiss.) Phitos et Damboldt.

The subdivision by Phitos and Damboldt was used in *Flora Europaea* (1972).

The *swainsonii* group is absent from southern Greece and instead the vicarious species *S. candida*, *S. chrysantha* and *S. spreitzenhoferi* occupy corresponding habitats. Their relationship to the *swainsonii* group was



investigated by means of crossing experiments and they will therefore be reviewed here.

Bory and Chaubard (1832) described *S. candida* from Mt. Taygetos (Diaforti, Thuria, Zarnate and Mistras). It was characterized by ovate to suborbicular leaves, a dense cottony white indumentum and white flowers.

*S. chrysantha* Boiss. et Heldr. was described in 1846 from Montis Maleva Laconia, near Agriani. It was closely related to *S. candida* but differed in its dense felted greyish white indumentum (not silky grey as in *S. candida*) and yellow flowers. In De Candolle's *Prodromus* (1848) Bentham regarded *chrysantha* as a variety of *S. candida*, but Boissier (1879) re-established it as a species. All subsequent authors have regarded these two taxa as separate species.

In his report from travels in the Ionian Islands Unger (1862) listed *S. candida* Borg. (sic!) from Cephaloniae, but Heldreich (1883), when referring to Unger's report, wrote "*S. candida* Bory et Chaub. ... in Cephalonia non vidi." He recorded *S. Swainsonii* Benth.  $\beta$ . *Scironica* Boiss. from six localities on Cephalonia and surmised that this was the plant Unger had seen.

Heldreich (1880) described *S. spreitzenhoferi* as a species closely related to *S. candida* but with a less dense indumentum particularly on the upper surface of the leaves, long petioles, and a corolla only half as large as that of *candida*.

Greuter and Rechinger (1967) pointed out the close relationship between *S. spreitzenhoferi*, *S. candida* and *S. chrysantha* and remarked upon the unusually wide range of variation within the three species in leaf size, indumentum, size of calyx and corolla. They also focused their attention on a *spreitzenhoferi*--like population on the Monemvasia Peninsula and suggested that local introgression had occurred in which *S. spreitzenhoferi* and some glabrous species (*S. messeniaca* Boiss.?) had taken part.

Flora Europaea (1972) recognized the three taxa as separate species.

Taxa recognized in this investigation. Abbreviations used in the text in parenthesis.

|                                |                    |                       |                            |
|--------------------------------|--------------------|-----------------------|----------------------------|
| S. swainsonii ssp. swainsonii  | (ssp. swainsonii)  | S.<br>swain-<br>sonii | The<br>swainsonii<br>group |
| S. swainsonii ssp. scyronica   | (ssp. scyronica)   |                       |                            |
| S. swainsonii ssp. melangavica | (ssp. melangavica) |                       |                            |
| S. swainsonii ssp. argolica    | (ssp. argolica)    |                       |                            |
| S. spruneri                    | (spruneri)         |                       |                            |
| S. ionica                      | (ionica)           |                       |                            |

S. euboica (euboica)

|                                         |                        |                                 |                         |
|-----------------------------------------|------------------------|---------------------------------|-------------------------|
| S. candida                              | (candida)              | S.<br>spreit-<br>zen-<br>hoferi | The<br>candida<br>group |
| S. chrysantha                           | (chrysantha)           |                                 |                         |
| S. spreitzenhoferi ssp. spreitzenhoferi |                        |                                 |                         |
|                                         | (ssp. spreitzenhoferi) |                                 |                         |
| S. spreitzenhoferi ssp. virella         | (ssp. virella)         |                                 |                         |

The swainsonii group here includes S. swainsonii, S. spruneri and S. ionica and the candida group S. candida, S. chrysantha and S. spreitzenhoferi.

## MORPHOLOGICAL NOTES

All taxa in this investigation are suffruticose perennials, usually branched and woody at the base with herbaceous erect or ascending flowering shoots.

Indumentum. The indumentum consists of short 1-celled hairs, up to 0.6 mm, and longer 1-celled eglandular hairs, usually 1--3.5 mm long, and sessile and stalked glands. The short hairs are straight or tomentose, the longer ones straight, appressed or wooly. All hairs are simple, usually arising from one basal cell, sometimes from a few. The chemical content of the glands evidently varies because the <sup>+</sup> noticeable smell is specific to each taxon.

Leaves. The lower leaves are petiolate in all taxa, the middle and upper ones petiolate, subsessile or sessile. Leaf size and shape are extremely variable and is often environmentally influenced. Also, seasonal differences can be noticed; spring and early summer leaves are larger than those produced later in the season. The outline of the shape is described according to the Systematics Association: Descriptive Terminology Chart 1a (Taxon 11, 1962). Fig. 2 shows part of the chart with the shapes used for the descriptions. The figure on the chart most closely corresponding to a particular leaf outline is referred to by its number in parenthesis after the descriptive terms e.g. ovate (S.A. 39), narrowly elliptic (S.A. 2). This relates to the general leaf shape and may have to be modified somewhat at base and apex (available in the description).

Inflorescence. The inflorescence is a terminal <sup>+</sup> dense spike of 2--6(--10)-flowered verticillasters; the lowest verticillasters are often somewhat distant. The floral leaves are largest in the lowest verticillaster and decrease in size upwards. The pedicels are less than 2(--3) mm except in *ionica* (2--4 mm).

Calyx. All taxa have subcampanulate calyces with 5 triangular teeth, equal or almost equal with the two lower teeth slightly shorter than the upper three of



## SYSTEMATICS ASSOCIATION : DESCRIPTIVE TERMINOLOGY

## CHART 1a

Symmetric Plane Figures

|                                               | 1                                                                                   | 2<br><i>anguste</i>                                                                 |                                                                                     | 3                                                                                                                                                                                     | 4<br><i>late</i>                                                                     | X<br>↓                                                                                | 5<br><i>transverso late</i>                                                           |  |
|-----------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|--|
|                                               | 12:1                                                                                | 6:1                                                                                 | 3:1                                                                                 | 2:1      3:2                                                                                                                                                                          | 6:5                                                                                  | 1:1                                                                                   | 5:6                                                                                   |  |
| A <i>ellipticus</i>                           |                                                                                     |    |    |  <i>ellipticus</i>  |    |    |    |  |
| B <i>oblongus</i><br><i>vel rectangularis</i> |    |    |    |                     |    |    |    |  |
| D <i>ovatus</i>                               |                                                                                     |    |    |                     |    |    |    |  |
| E <i>obovatus</i>                             |                                                                                     |   |   |                   |   |   |   |  |
| H <i>triangularis</i>                         |  |  |  |                 |  |  |  |  |

Fig. 2. Part of the S.A. chart 1a used for leaf outlines in the descriptions. From the Systematics Association Committee for descriptive Biological Terminology in Taxon 11, 1962.

which the middle tooth sometimes is slightly larger. Calyx and corolla were measured at anthesis. Later the calyx increases in the size considerably. The ratio corolla tube: calyx is very variable within populations. In some taxa, however, the calyx is always noticeably shorter than the corolla tube (ssp. *scyronica*, *spreitzenhoferi* and *tetragona*).

**Corolla.** The upper lip of the corolla is erect, entire, slightly concave and  $\frac{+}{-}$  hairy on the outside. The middle lobe of the lower lip is  $\frac{+}{-}$  folded downwards lengthwise, or spreading. The angle of the lower lip was used as a character for distinguishing taxa within *S. swainsonii* and was measured as in Fig. 13, p. 66. The corolla tube is always annulate within at about the middle; the annulus is transverse in all taxa except in *candida*, *chrysantha* and *spreitzenhoferi*, where it is oblique, and in *ionica* where it is transverse to somewhat oblique.

**Stamens.** The filaments are hairy in all taxa and at least the anterior pair is coloured (not in *euboica*).

**Fruit.** The nutlets are obovoid, dark brown to brownish black, ranging in size from c. 2.2 x 1.6 mm to c. 1.6 x 1.4 mm. *Spreitzenhoferi* has the smallest nutlets and the pericarp is differently sculptured (Fig. 3).

**Distribution and habitat.** All species in this investigation are chasmophytes in calcareous habitats, each limited to a particular area in southern and central Greece. Only a few times have two species been found growing in the immediate vicinity of each other (*candida* and *messeniacae*; *chrysantha* and ssp. *virella*).

Figs. 4, 5 and 6 show the distributions. Vegetative propagation has not been observed. The plants are not eaten by grazing animals.

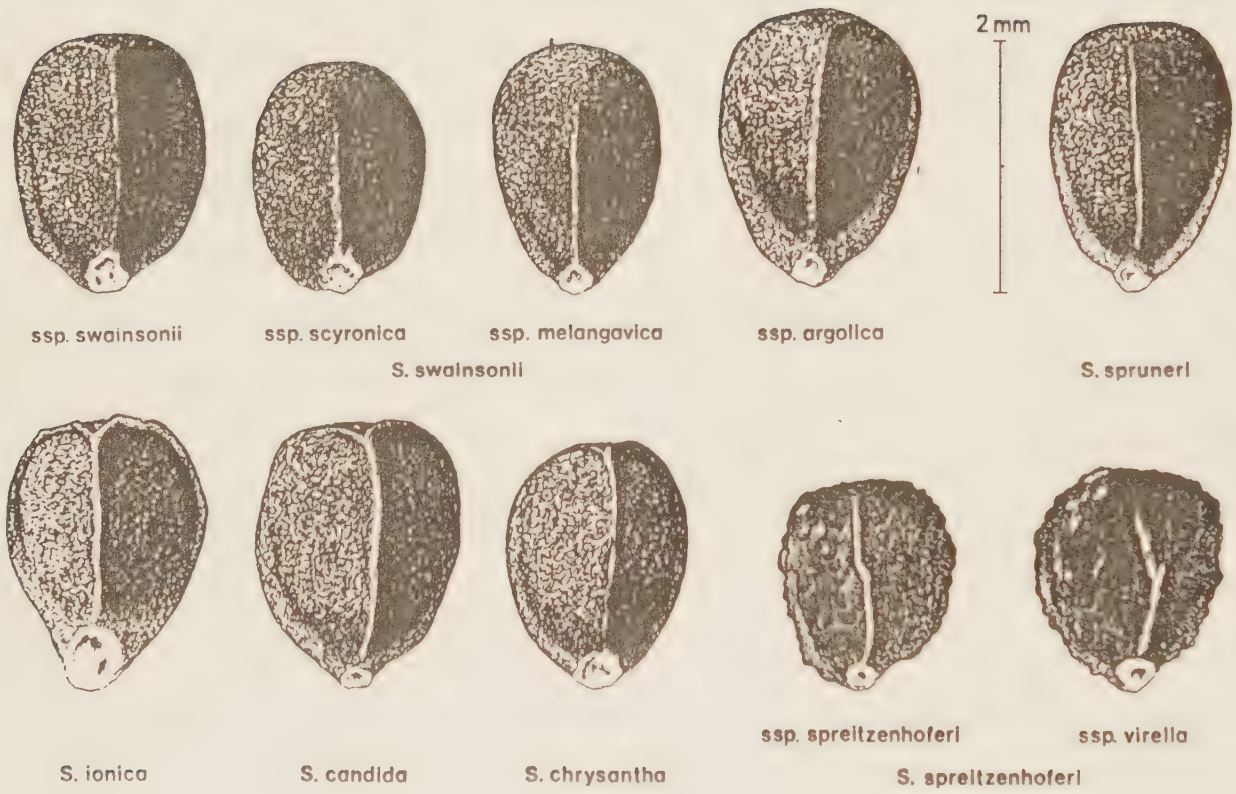


Fig. 3. Nutlets.



Fig. 4. Distribution of *S. swainsonii* ssp. *swainsonii* ●, -- ssp. *scyronica* ★, -- ssp. *melangavica* ▲, -- ssp. *argolica* ■, -- *S. spruneri* ○, -- *S. ionica* □.



Fig.5. Distribution of *S. candida* ●, -- *S. chrysantha* ▲, -- *S. spreitzenhoferi* ssp. *spreitzenhoferi* ■, -- ssp. *virella* ★.



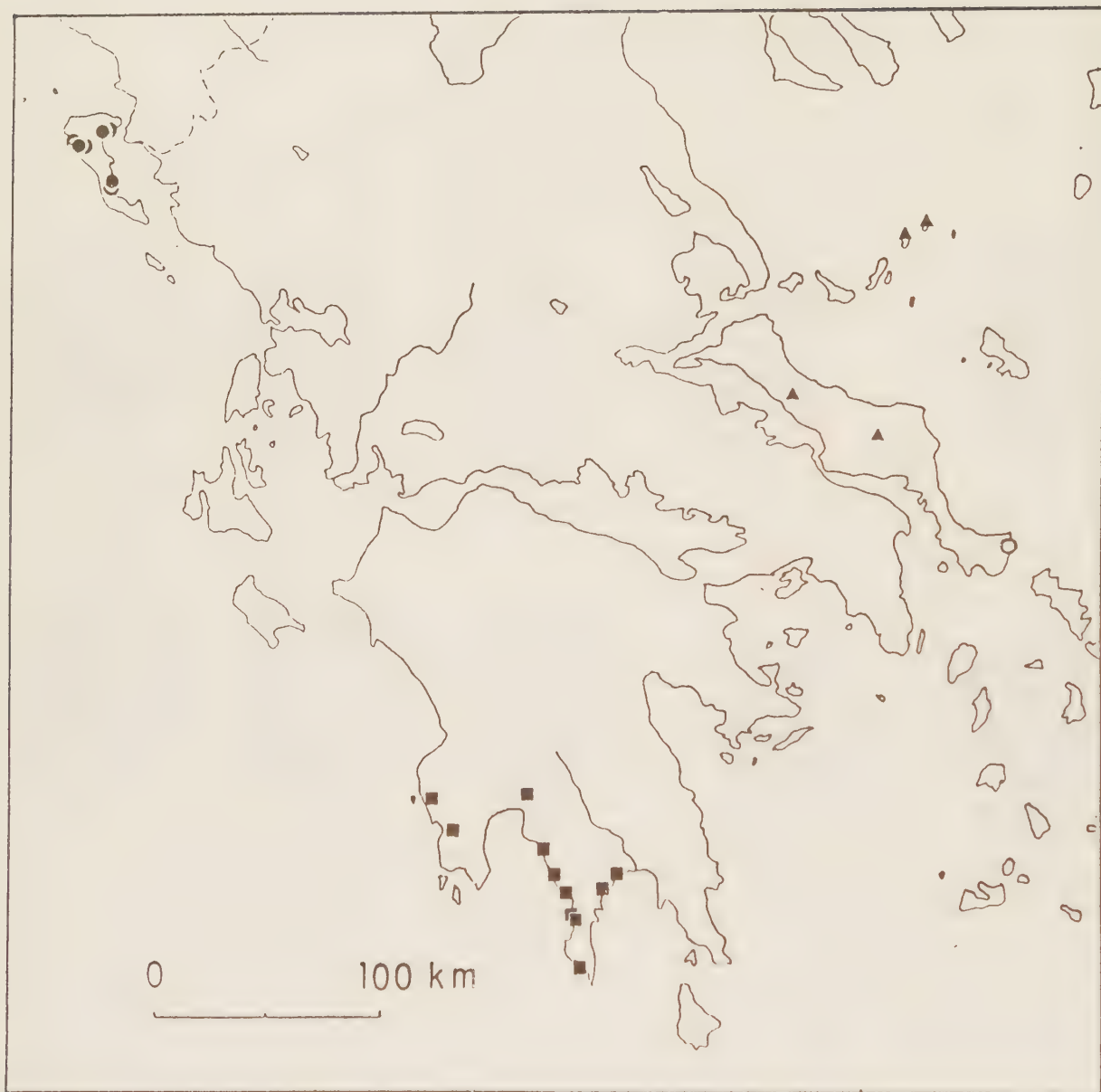


Fig. 6. Distribution of *S. mollissima* ●, -- *S. tetragona* ▲, -- *S. euboica* ○, -- *S. messenica* ■.

## TAXONOMY

In addition to taxa of the *swainsonii* and *candida* groups four other species are described here since they were used in the crossing experiments:

*S. euboica* Rech. f., *S. messeniaca* Boiss., *S. mollissima* Willd., and *S. tetragona* Boiss. et Heldr.

## KEY TO THE SPECIES

- |                                                                                             |                           |
|---------------------------------------------------------------------------------------------|---------------------------|
| 1. Flowers yellow or yellowish                                                              | 2                         |
| -- Flowers white                                                                            | 3                         |
| 2. Leaves densely lanate, eglandular                                                        | <i>S. chrysantha</i>      |
| -- Leaves sparsely hairy, glandular                                                         | <i>S. messeniaca</i>      |
| 3. Calyx teeth emucronate                                                                   | 4                         |
| -- Calyx teeth mucronate                                                                    | 5                         |
| 4. Internodes long (5--10 cm); lowest floral leaves equal to<br>or shorter than calyx       | <i>S. tetragona</i>       |
| -- Internodes short; lowest floral leaves longer than calyx                                 | <i>S. spreitzenhoferi</i> |
| 5. Upper corolla lip with purplish red markings or entire<br>upper corolla lip purplish red | 6                         |
| -- Upper corolla lip without purplish red markings                                          | 8                         |
| 6. Upper corolla lip 5--7 mm, lower corolla lip 10--14 mm;<br>floral leaves petiolate       | <i>S. candida</i>         |
| -- Upper corolla lip 3.5--5 mm, lower corolla lip 7.5--11 mm;<br>floral leaves sessile      | 7                         |

7. Verticillasters 2-flowered; upper corolla lip with purple  
 markings *S. swainsonii*
- Verticillasters (4--6)-flowered; upper corolla lip purplish  
 red or with purplish red markings *S. spreitzenhoferi*
8. Lower floral leaves petiolate 9
- Lower floral leaves sessile or subsessile 10
9. Leaves greyish to greyish green; verticillasters 2(4--6)-  
 -flowered *S. ionica*
- Leaves green; verticillasters 6-flowered *S. mollissima*
10. Calyx length 4--6.5(--7) mm; leaves broadly ovate or broadly  
 ovate--broadly triangular, base truncate or subcordate  
*S. spreitzenhoferi*
- Calyx length 7--12 mm; leaves rarely broadly ovate,  
 base obtuse 11
11. Plants densely glandular and sticky; tufts of long  
 hairs between calyx teeth *S. euboica*
- Plants <sup>+</sup> glandular but not sticky; without tufts of long  
 hairs or with only few long hairs between calyx teeth 12
12. Calyx with sessile and stalked glands; upper floral leaves  
 elliptic, narrowly elliptic or ovate *S. swainsonii*
- Calyx with sessile glands only; upper floral leaves ovate-  
 -triangular *S. spruneri*

*S. swainsonii* Benth.

Bentham 1834 p. 535.

Orig. coll. Greece. Parnassus W. Swainson *Stachys* ? 64 (K, lectotype).

Flowering shoots 7--25(--40) cm. Indumentum <sup>+</sup> sericeous, hirsute or tomentose, appressed on leaves; <sup>+</sup> patent or tomentose on stem; consisting of short and long eglandular hairs, sessile and stalked glands. Petiole of lower leaves 3--18 mm, petioles gradually decreasing in size upwards, upper leaves sessile. Leaf lamina narrowly ovate, ovate, broadly ovate, narrowly elliptic, elliptic, broadly elliptic, narrowly elliptic--narrowly oblong or obovate (S.A. 37, 38, 39, 40; 2, 3, 4, 5; 2--14; 47, 48); greyish green, green or yellowish green; prominently net-veined; base cuneate, obtuse or attenuate; apex obtuse or acute; margin crenate, serrate or dentate; in lower leaves 6--25 x 3--20 mm, in middle 7--30 x 4--16 mm and in upper 8--33 x 4--20. Verticillasters 2--6(--10)-flowered, 2--5(--8) per inflorescence. Floral leaves sessile, lowermost like cauline leaves; middle and upper elliptic or narrowly elliptic (S.A. 2, 3, 4), gradually smaller; lowest floral leaves narrowly--broadly elliptic, oblong, ovate or obovate; lamina 6--28 x 4--15 mm; number of teeth 3--9, on second floral leaves 1--8. Bracteoles linear (S.A. 12), 0.5--11 mm long or lacking. Calyx 5.5--12 mm; lower half of calyx hairy inside; middle tooth 1.7--4.6 x 1.2--2.7 mm; mucro up to 0.6 mm. Corolla white with a purplish red pattern on lower lip, upper lip with or without markings; upper lip 3.5--7.5 mm, lower lip 7.5--14.5 mm, angle 125--180°; corolla tube 7--12 mm. 2n=34.

Flowering time. April, May, June.

Distribution. Mt Parnassos, Isthmus of Corinth, Argolis.

Note. In his description of *S. swainsonii* Bentham mentioned *Prasium hirsutum* as a synonym. However his statement is not affirmative; he restricted himself by writing 'fide exempl. in herb. Vahl. sub nomine *Prasii minoris*'.



The specimen he referred to in the Vahl herbarium (C) is not the same as the material from Parnassos collected by Swainson and on which Bentham based his description and which he named in honour of the collector. The Parnassos specimen available from the Hooker herbarium, Kew Gardens, agrees with Bentham's diagnosis for *S. swainsonii* and not with the description of *Prasium hirsutum*. The name *S. swainsonii* can therefore not be considered a 'nomen novum', since it is not 'an avowed substitute' as required in the International Code of Nomenclature, Article 7.4. As a consequence the type for *Prasium hirsutum* does not automatically become the type for *S. swainsonii* and the Parnassos specimen (K) can therefore be selected as lectotype for Bentham's description. Bentham also mentioned Ithaca as a locality according to information from Webb and a specimen in Sibthorp and Hooker's herbarium. The Ithaca plant differs somewhat and later the populations on the Ionian Islands were described as a separate species, *S. ionica* Halácsy (1908).

Greuter (1968) discussed a similar but less clear case concerning *Phaca alpina* L.

#### Key to the subspecies

- |                                                                  |                         |
|------------------------------------------------------------------|-------------------------|
| 1. Plants greyish green                                          | 2                       |
| -- Plants green or yellowish green                               | ssp. <i>argolica</i>    |
| 2. Upper lip 3.5--6.5 mm, lower lip 7.5--10 mm, verticillasters  |                         |
| 2-flowered                                                       | 3                       |
| -- Upper lip 5--7.5 mm, lower lip 10.5--14.5 mm, verticillasters |                         |
| 2--6-flowered                                                    | ssp. <i>swainsonii</i>  |
| 3. Calyx 5.5--7 mm, upper lip with purplish red markings         | ssp. <i>scyronica</i>   |
| -- Calyx 7--11 mm, upper lip without markings                    | ssp. <i>melangavica</i> |

Note. Occasionally *ssp. swainsonii*, and rarely *ssp. scyronica* and *ssp. melangavica* may be less hairy and look more green than greyish green (shade plants in particular). Also, *ssp. argolica* may be hairier than usual and approach a greyish green colour. *Ssp. scyronica* and *ssp. melangavica* can be distinguished by their small flowers, but to distinguish between *ssp. swainsonii* and *ssp. argolica* is more difficult. *Ssp. argolica* usually has a sturdier habit, an indumentum of proportionally fewer short hairs than that of *ssp. swainsonii*, often more flowers per verticillaster (4--10, *ssp. swainsonii* 2--6), the leaf margin is oftener dentate than serrate and the bracteoles variable but often longer (up to 11 mm, *ssp. swainsonii* up to 3,5 mm). Cuneate leaf bases occur in both subspecies but attenuate leaf bases are also common in *ssp. argolica*, not in *ssp. swainsonii* where the alternative is obtuse leaf bases.

*Ssp. swainsonii*, Figs. 7 A and 8

*S. swainsonii* Benth.  $\alpha$ . [var.] *typica* Hal., Halácsy 1908 p. 85 (nom. illeg.).

Flowering shoots 10--25(--40) cm. Indumentum sericeous, appressed, glandular; leaves covered with short and less numerous long hairs. Petiole of lower leaves 7--18 mm. Leaf lamina ovate, occasionally broadly ovate, or obovate (S.A. 38, 39, 40; 47, 48), becoming elliptic or broadly elliptic (S.A. 3, 4, 5) upwards, greyish green, not shiny; base cuneate or obtuse; apex obtuse or acute; margin serrate; in lower leaves 10--20 x 5--11 mm, in middle 11--25(--30) x 6--15 mm, in upper 13--29(--32) x 7--16(--20) mm. Verticillasters 2--6-flowered. Lower floral leaves elliptic (S.A. 3; 2--3, 3--4, 4), lamina 11--27 x 6--14 mm; number of teeth 5--9; on second floral leaves 3--7.

Bracteoles up to 3.5 mm. Calyx 7--12 mm, middle tooth 2.1--4.2 x 1.4--2.7 mm. Upper corolla lip without markings, 5--7.5 mm; lower lip 10.5--14(--14.5) mm, angle (126--136--140(--148))°; corolla tube 8--12 mm. 2n=34.



Fig. 7. *S. swainsonii*. -- A: *ssp. swainsonii*. -- B: *ssp. scyronica*. -- C: *ssp. melangavica*. -- D: *ssp. argolica*.



Fig. 8. Flowers.



Flowering time. May, June.

Distribution. Mt Parnassos, Mt Lidorikióu.

Ssp. *scyronica* (Boiss.) Phitos et Damboldt. Figs. 7 B and 8

Phitos & Damboldt 1969 p. 600.

*S. scyronica* Boiss., Boissier 1844 p. 31. -- *S. swainsonii* Benth.  $\beta$ . [var.]  
*scyronica* (Boiss.) Boiss., Boissier 1879 p. 735. -- *S. swainsonii* Benth.  $\beta$ .  
 [forma] *scyronica* (Boiss.) Hay., Hayek 1929 p. 298.

Orig. coll. Kaki Skala inter Megara et Corinthe [in fissuris rupium *Scyroni-*  
*carum* prope Isthmus Corinthiacum ubi Aprili 1842] Boissier (G-Boiss., holo-  
 type).

Flowering shoots 7--15 cm. Indumentum sericeous, appressed, on lower part of  
 stem sometimes  $\frac{+}{-}$  tomentose, glandular. Petiole of lower leaves 3--11 mm.  
 Leaf lamina elliptic (S.A. 3, 4); greyish green to slightly greyish green,  
 appearing somewhat shiny owing to long appressed hairs; base cuneate or  
 obtuse; apex obtuse or acute; margin crenate; in lower leaves 6--10(--13) x  
 4--6 mm, middle (7--9--13(--16) x 4--8 mm, upper 8--12(--14) x 4--7 mm.  
 Verticillasters 2-flowered. Lowest floral leaves elliptic (S.A. 3, rarely  
 2--3 or 4); lamina 6--11 x 4--6 mm, number of teeth 3--6; on second floral  
 leaves 2--4. Bracteoles about 0.5 mm long. Calyx 5.5--7 mm; middle tooth  
 1.9--2.9 x 1.2--2.0 mm. Upper corolla lip with purplish red (deep magenta)  
 markings, 3.5--5 mm, lower lip 7.5--10 mm; angle 170--180°; corolla tube  
 7--9 mm. 2n=34.

Flowering time. April, May.

Distribution. The Scyronides Rock (Kaki Skala) near Megara on the southeast  
 side of the Isthmus of Corinth.

*Ssp. melangavica* D. Perss. ssp. nov., Figs. 7 C and 8

Differt a *ssp. swainsonii* praecipue floribus parvis, indumento denso e ciliis plerumque longis formato, foliis supremis et mediis anguste ellipticis, foliis floralibus anguste ellipticis apice acuto et margine sparse serrato praeditis.

Orig. coll. Korinthia. 11 km NNW of Loutraki, rocks facing S, c. 190 m.

May 15 1976. D. Persson, UC 122 (LD, holotype).

Flowering stems 8--15(--20) cm. Indumentum  $\pm$  sericeous, more long hairs than short,  $\pm$  appressed on leaves,  $\pm$  patent on stem, glandular. Petioles of lower leaves 3--12 mm. Leaf lamina, in lower leaves narrowly ovate, sometimes obovate (S.A. 37, 46--47), in middle and upper narrowly elliptic or narrowly elliptic--narrowly oblong (S.A. 2, 2--14); greyish green, somewhat shiny owing to long appressed hairs; base cuneate, in upper leaves attenuate; apex acute, in lower leaves obtuse; margin serrate; in lower leaves 7--18 x 3--9 mm, in middle 11--24 x 4--11 mm, in upper 11--25 x 5--12 mm. Verticillasters 2-flowered, occasionally 3--6-flowered. Lowest floral leaves narrowly elliptic or occasionally narrowly elliptic to narrowly oblong (S.A. 2, 2--3, 2--14), lamina 12--23 x 3--10 mm, number of teeth 3--6; on second floral leaves 1--3. Bracteoles 0--2.5 mm long. Calyx 7--11 mm; middle tooth 1.9--3.9 x 1.3--2.2 mm. Upper corolla lip without markings, 4--6.5 mm, lower lip 7.5--10(--11) mm; angle (146--157--168(--180))<sup>0</sup>; corolla tube 8--10 mm.  $2n=34$ .

Flowering time. April, May.

Distribution. Isthmus of Corinth from Loutraki to Akr. Melangavi.

Note. *Ssp. melangavica* was previously included in *ssp. swainsonii* (Halácsy 1908 p. 85), and *ssp. scyronica* (Boissier 1879 p. 735, Boiss., Heldr., Orph. Fl. Gr. Exs. 438).

*Ssp. argolica* (Boiss.) Phitos et Damboldt, Figs. 7 D and 8

Phitos & Damboldt 1969 p. 600.

*S. argolica* Boiss., Boissier in De Candolle 1848 pp. 488--489. -- *S.*

*swainsonii* Benth. γ. [var.] *argolica* (Boiss.) Boiss., Boissier 1879 p. 735.

Orig. coll. Palamidio [Naupliam Argolides] Spruner 1841 (G-Boiss., lectotype).

Flowering shoots 9--25(--35) cm. Indumentum hirsute, of more long hairs than short, patent on stem, patent to  $\frac{+}{-}$  appressed on leaves, glandular. Petioles of lower leaves 8--18 mm. Leaf lamina elliptic or narrowly elliptic, rarely obovate (S.A. 3, 2, 47); green, sometimes yellowish green; base attenuate or cuneate; apex acute or obtuse; margin dentate or serrate; in lower leaves 12--25 x 4--13(--20) mm, in middle 13--28 x 5--16 mm, in upper 13--33 x 4--14 mm. Verticillasters 4--6(--10)-flowered. Lowest floral leaves narrowly elliptic to elliptic, narrowly elliptic to narrowly oblong, narrowly ovate, ovate, occasionally ovate--triangular or obtuse (S.A. 2, 2--3, 3, 3--4; 2--14; 37, 37--38, 38, 38--75; 46); lamina 11--28 x 4--15 mm; number of teeth 4--9, on second floral leaves (2--)4--6(--8). Bracteoles 0--11 mm long. Calyx 7--12 mm, middle tooth 1.7--4.6 x 1.4--2.7 mm. Upper corolla lip without markings, 4.5--6.5 mm; lower lip 8.5--13 mm, angle 125--146°; corolla tube 9--12 mm.  $2n=34$ .

Flowering time. April, May.

Distribution. Argolis, Idhra.

Note. *Ssp. argolica* is extremely variable and there are plants which in certain characters e.g. indumentum and leaf shape resemble *ssp. swainsonii* or *sprunerii* (and *ssp. melangavica*--at Akrokorinth). The variation was analysed by statistical methods (see Morphological variation).

*S. spruneri* Boiss., Figs. 9 A and 8

Boissier in De Candolle 1848 p. 488.

Orig. coll. *Stachys spruneri*! Boiss. rochers du pied du Parnes près Mynidi (G, lectotype).

Flowering shoots, 11--35 cm. Indumentum mainly of hispid hairs, patent on stem, patent to appressed on leaves, and sessile glands. Stalked glands lacking or rare. Petiole of lower leaves 3--13 mm, upper leaves sessile. Leaf lamina narrowly elliptic to narrowly oblong (S.A. 1--2, 2; 13--14); green or yellowish green; base attenuate in lower leaves, obtuse in upper leaves; apex acute; margin serrate; in lower leaves 10--19(--34) x 4--10(--13) mm, in middle 23--36(--47) x 4--9(--17) mm, in upper 14--28(--44) x 4--10(--15) mm. Verticillasters (4--6(--8)-flowered, 2--5(--8) per inflorescence. All floral leaves sessile; all ovate--triangular (S.A. 38--75) or lowest pair elliptic--oblong (S.A. 2--14), second and upper ovate (S.A. 38) with truncate base and acute apex; lamina in lowest floral leaves 11--33(--45) x 4--12(--16) mm. Bracteoles linear (S.A. 12) up to 5 mm long. Calyx 8--11 mm, lower half of calyx hairy inside; middle tooth 2--4 x 1.5--2.5 mm; mucro 0.5--1 mm. Corolla white with purplish red pattern on lower lip, upper lip without markings; upper lip 4--6.5 mm; lower lip 10--12 mm, angle 121--135°; corolla tube 8--12 mm.  $2n=34$ .

Flowering time. April, May.

Distribution. Attiki, Boiotia, Korinthia.





Fig. 9. -- A: *S. spruneri*. — B: *S. ionica*.

*S. ionica* Hal., Figs. 9 B and 8

Halácsy 1908 p. 85.

Orig. coll. Graecia: Zante. 9 April 1902. Sterneck, iter graeco-turcicum no. 408 (W, lectotype).

*S. ionica* Hal. / $\beta$ . [var.] *cephalonica* Hal., Halácsy 1908 p. 85.

Orig. coll. In rupestribus calcareus Cephalonia. Unger (W, holotype).

Flowering shoots 9--20(--30) cm. Indumentum dense, tomentose, on upper surface of leaves also sericeous; sessile glands on stem and leaves, on calyx <sup>+</sup> sessile glands and moderate to sparse or entirely lacking stalked glands. All leaves petiolate; petioles of lower leaves 8--13 mm. Leaf lamina ovate or broadly ovate or elliptic (S.A. 39, 40, 41; 3--4); greyish to greyish green; base obtuse or truncate, in upper leaves attenuate; apex obtuse or rounded; margin crenate; in lower leaves 8--14(--18) x 5--12 mm, in middle 8--19(--28) x 5--15(--22) mm, in upper 7--18(--26) x 5--15(--18) mm. Verticillasters 2(4--6)-flowered, 2--5(--8) per inflorescence. Floral leaves petiolate, ovate or elliptic (S.A. 38, 39; 3) with obtuse or attenuate base; lamina in lowest floral leaves 7--22(--25) x 4--19 mm. Bracteoles linear or elliptic (S.A. 12, 1), (4--)6--9(--12) mm long. Calyx (7--)9--10(--11) mm; lower half of calyx glabrous inside; middle tooth 2--3 x 1.5--2(--2.5) mm; mucro c. 0.5 mm. Corolla white with a greyish magenta pattern on lower lip or middle lobe entirely tinged with rose; upper lip without markings; upper lip 5--7 mm; lower lip 10--13 mm; angle c. 170°; corolla tube 9--11 mm. 2n=34.

Flowering time. April, May(--June).

Distribution. The Ionian Islands.

Note. When Halácsy described *S. ionica* he also recognized  $\beta$ . [var.] *cephalonica* which was distinguished by its glandular calyx. All plants observed in the field had  $^+$  sessile glands, but only some had stalked glands. Plants with and without stalked glands usually occur in varying proportions within the same population, e.g. on Cephalonia at Assos and Poros and on Leucas at Vassiliki and Cape Tsouanna. Since they seemingly have no other distinguishing characters the glandular and eglandular plants do not merit taxonomic recognition.

*S. candida* Bory et Chaubard, Figs. 10 A and 8

Bory & Chaubard 1832 pp. 167--168.

Orig. coll. *Stachis candida* Bory et Chaub. (Chaubard scrpt.) Morée/Chaub.  
(P, lectotype).

Flowering shoots, 9--22(--30) cm. Indumentum dense, tomentose-lanate and sericeous, sessile and stalked glands lacking or very sparse, when present, on calyx only. All leaves petiolate, petiole of lower leaves 7--13 mm. Leaf lamina broadly ovate, circular or transversely elliptic (S.A. 40, 41; 6, 7) whitish  $^+$  shining; base truncate, obtuse or rarely subcordate; apex rounded or obtuse; margin crenate; in lower leaves (7--15--22(--25) x (5--13--17(--21) mm, in middle (12--19--27 x (14--16--25 mm, in upper 17--23(--31) x 13--23(--30) mm. Verticillasters (2--6)-flowered 2--5(--8) per inflorescence. Floral leaves petiolate, lower like cauline leaves, upper elliptic with attenuate base; lamina in lowest floral leaves (9--14--21(--25) x (8--13--23(--27). Bracteoles linear (S.A. 12) up to 1 mm long. Calyx 7.5--10.5(--11) mm; lower half of calyx glabrous inside, middle tooth 2.5--3.5 x 1.5--2 mm; mucro 0.5--1(--1.5) mm. Corolla

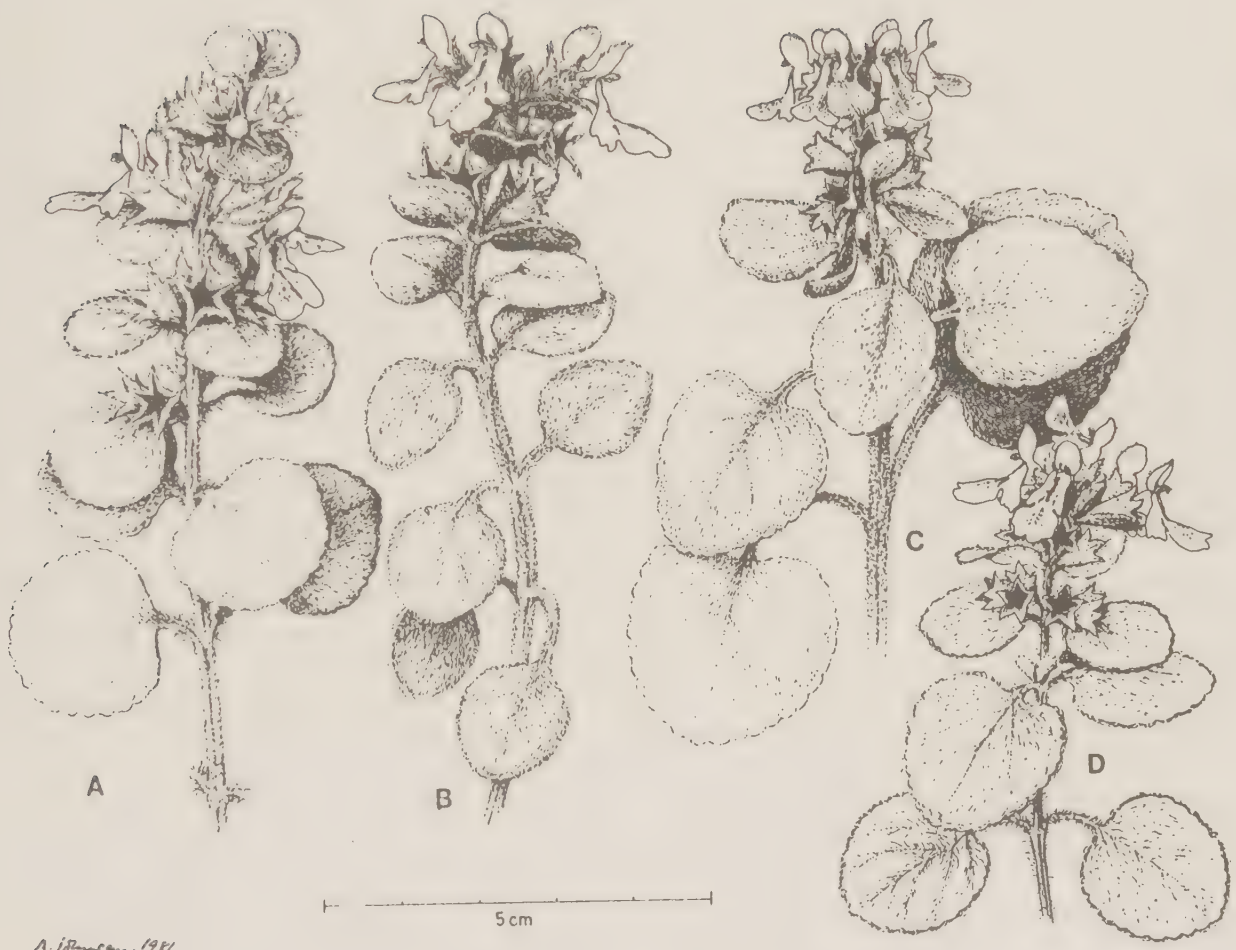


Fig. 10. -- A: *S. candida*. -- B: *S. chrysantha*. -- C: *S. spreitzenhoferi* ssp. *spreitzenhoferi*. -- D: *S. spreitzenhoferi* ssp. *virella*.



white with a purplish red pattern on lower lip, entire middle lobe often pale purplish pink, upper lip white with purplish red markings; upper lip 6--7 mm, lower lip 11--13(--14) mm; corolla tube 9--11 mm.  $2n=34$ .

Flowering time. May, June.

Distribution. Mt Taiyetcs.

*S. chrysantha* Boiss. et Heldr., Figs. 10 B and 8

Boissier 1846 p. 56.

Orig. coll. Ad rupes village d'Agriani aux pentes lacedemoniennes de la chaîne de Malevo. 1 juillet 1844. [Heldreich] 203 (G-Boiss., holotype).

Flowering shoots 10--25 cm. Indumentum dense, lanate; glands lacking or sparse, when present, on calyx only. All leaves petiolate, petioles of lower leaves 6--15 mm. Leaf lamina broadly ovate, broadly elliptic or circular (S.A. 39--40, 40, 41; 5, 6); greyish white not shining; base truncate or obtuse rarely subcordate; apex rounded or obtuse; margin crenate; in lower leaves (8--12--20 x (8--10--17(--20) mm, in middle 15--26(--31) x 12--24 mm, in upper 13--25 (--34) x 12--20(--24) mm. Verticillasters 2--6-flowered, 2--5(--8) per inflorescence. Floral leaves petiolate, ovate with truncate or obtuse base; only uppermost elliptic; lamina in lower leaves (9--11--24(--34) x (8--11--23(--28). Bracteoles linear (S.A. 12), up to 2 mm long. Calyx 8--10(--12) mm; lower half of calyx glabrous inside; middle tooth 2--3.5(--4) x 1.5--2.5 mm; mucro 0.5--1(--1.5) mm. Corolla yellow with a purple pattern on lower lip, upper lip with or without purple markings; upper lip 6--7 mm; lower lip 11--13 mm; corolla tube 8--10 mm.  $2n=34$ .

Flowering time. May, June.

Distribution. Mt Parnon, Malëa Peninsula.

*S. spreitzenhoferi* Heldr.

Heldreich 1880 p. 344.

Orig. coll. Greece. Cerigo. An den Felsenwänden beim Eingange in die Grotto Hippopotamo. 19. Juni 1880 leg. G.C. Spreitzenhofer (W, lectotype).

Flowering shoots 10--23 cm. Indumentum lanate, lanate--tomentose, sericeous, patent, glandular (stalked and sessile glands). Petiole of lower leaves (5--10--22 mm; middle and upper leaves petiolate. Leaf lamina broadly ovate, or broadly ovate--broadly triangular (S.A. (39), 40, 41, 40--77); greyish green or green; base truncate or subcordate; apex obtuse or rounded; margin crenate or crenulate; in lower leaves (7--11--23(--31) x 6--19(--22) mm, in middle 13--27(--38) x 11--27(--33) mm, in upper 10--28(--35) x 8--25(--27) mm. Verticillasters (4--6)-flowered, 3--5(--8) per inflorescence. Floral leaves subsessile, elliptic (S.A. 2) decreasing in size upwards; lamina of lowest floral leaves 9--20(--22) x 6--15(--20) mm. Bracteoles linear (S.A. 12), 1--3(--4) mm long. Calyx with mucronate or emucronate broadly triangular (S.A. 77, 78, (79)) teeth; lower half of calyx shortly hairy inside; 4--6.5(--7) mm; middle tooth 1--2.5 mm x 1--2.5 mm. Corolla white with dark purplish red pattern on lower lip, upper lip white or sometimes dark purplish red, or white with purplish red markings; upper lip 3.5--4.5(--5) mm; lower lip 8--11 mm; corolla tube 7.5--10.5 mm.  $2n=34$ .

Flowering time. May, June.

Distribution. Kithira, Neapolis, Monemvasia.

#### Key to the subspecies

1. Plants whitish grey, densely lanate, lanate--tomentose, glandular;

floral leaves eglandular, densely lanate below, densely sericeous

below.

ssp. spreitzenhoferi

-- Plants green or greyish green, sparsely (---moderately) sericeous, sericeous---tomentose, (or lanate), densely glandular; floral leaves glandular, sericeous (---tomentose) below, sericeous above.

ssp. virella

Ssp. spreitzenhoferi, Figs. 10 C and 8

Flowering shoots 10--20 cm. Indumentum on stem dense, lanate---tomentose, glandular (sessile glands); on lower surface of leaves densely lanate or tomentose, <sup>+</sup> glandular (sessile glands); on upper surface less dense, sericeous and glandular (sessile glands); calyx densely to sparsely hairy with long and short hairs, eglandular or sparsely to moderately glandular (sessile and stalked glands); floral leaves eglandular, or with sparse sessile glands and no stalked glands, densely lanate below, densely sericeous above. Petioles of lower leaves 12--22 mm. Leaf lamina broadly ovate or broadly ovate---broadly triangular (S.A. (39) 40; 40--77), greyish green above, whitish below; base truncate or subcordate; apex obtuse or rounded; margin crenate or crenulate; in lower leaves 13--23(---31) x 10--19(---22) mm in middle 16--25(---38) x 14--25(---33) mm, in upper (11--17) 17--28(---35) x (8--12) 12--24(---27) mm. Lamina of lowest floral leaves 11--20(---22) x 6--13(---16) mm. Calyx 4--6.5 mm; middle tooth 1--2.5 x 1--2.5 mm. Upper corolla lip 3.5--4.5 (---5) mm; lower lip 9.5--11 mm; corolla tube 9--10.5 mm. 2n=34.

Flowering time. May, June.

Distribution. Kithira, Neapolis.

Ssp. virella D. Perss. ssp. nov. Figs. 10 D and 8

Differt a ssp. spreitzenhoferi pilis sparsioribus, glandibus densioribus

stipitatis et sessilibus et colore plantae viridi, non albidocinereo.

Orig. coll. Lakonia. Monemvasia. Large vertical rock facing S, c. 50 m.

June 20 1973. D. Persson BE 37. (LD, holotype).

Flowering shoots 10--23 cm. Indumentum on stem moderately dense, lanate, glandular (sessile glands); leaves, on lower surface sparsely to moderately sericeous (--tomentose) and  $\frac{+}{-}$  patent, densely glandular (sessile glands), on upper surface sparsely (to moderately) sericeous, glandular (innumerable sessile glands); calyx sparsely to moderately hairy with mostly short patent hairs, numerous sessile and stalked glands; floral leaves glandular (sessile and stalked glands) sericeous (--tomentose) below, sericeous above. Petioles of lower leaves (5--10--20 mm. Leaf lamina broadly ovate (S.A. 40, 41) green or slightly greyish green; base truncate or subcordate; apex obtuse or rounded; margin crenate or crenulate; in lower leaves (7--11--21(--24) x 6--19(--21) mm, in middle 13--27(--34) x 11--27(--32) mm, in upper 10--27(--30) x 9--25(--27) mm. Lamina of lowest floral leaves 9--17(--19) x 6--15(--20) mm. Calyx 5.5--6.5(--7) mm, middle tooth 1--2 x 1--2 mm. Upper corolla lip 3.5--4.5 mm, lower lip 8--9(--10) mm, corolla tube 7.5--9.5 mm.  $2n=34$ .

Flowering time. May, June.

Distribution. Monemvasia.

*S. euboica* Rech. f.

Rechinger 1961 p. 391.

Orig. coll. Insula Euboea meridionalis: 5 km a promontorio Kaphireos meridiem versus in fissuris rupium calc. 22.VI 1958. K.H. Rechinger, Iter Graecum IX: no 19064 (W, holotype).



Flowering shoots 15--25 cm. Indumentum hirsute, with sparse long hairs and with innumerable strong-smelling, sticky, sessile and shortly stalked glands. Petioles of lower leaves 10--20 mm. Middle and upper leaves sessile. Leaf lamina narrowly obovate to obovate or elliptic (S.A. 46--47, 3, 4); green or yellowish green; base attenuate, cuneate or obtuse; apex obtuse; margin coarsely crenate-serrate; in lower leaves 20--30 x 10--16 mm, in middle 30--45 x 15--20 mm, in upper 24--35 x 14--19 mm. Verticillasters (4--6) 6--12-flowered, 1--3 per inflorescence. Floral leaves sessile, ovate or broadly ovate, ovate to triangular or elliptic (S.A. 39--40; 39--76; 4), acute with truncate base; lamina in lowest floral leaves 15--38 x 7--19 mm. Bracteoles narrowly elliptic (S.A. 1), 6--14 mm long. Calyx 10--12 mm; lower half of calyx sparsely hairy inside; middle tooth 3.5--5 x 2.5--3 mm; mucro 1--1.5 mm. Corolla white with a pale lilac pattern on lower lip, upper lip without markings; upper lip 6--7 mm, lower lip 12--13.5 mm. Corolla tube 10--12 mm.  $2n=34$ .

Flowering time. May, June.

Distribution. Euboea.

*S. messeniaca* Boiss.

Boissier in De Candolle 1848 p. 475.

Orig. coll. (Stachyotypus Bnth.) *Stachys messeniaca* Bssr. -- *Tournefortiana* Chb. (Non Poir.) Morée, Coll.<sup>n</sup> Bory de St. Vt. (P, lectotype).

Flowering shoots 23--55 cm. Indumentum of sparse short and long patent hairs and numerous sessile and stalked glands; on upper surface of leaves few mainly short hairs. Petioles of lower leaves 12--22(--30) mm, upper leaves

subsessile. Leaf lamina ovate (S.A. 38--39, 39); green; base truncate or cordate; apex obtuse or acute; margin crenate; in lower leaves 13--27(--48) x 11--20(--32), in middle 18--40(--55) x 11--30(--40), in upper 13--37(--46) x (8--14)14--26. Verticillasters 6(--10)-flowered, 6--12(--15) per inflorescence. Floral leaves sessile except lowest pair, subsessile, lamina (7--17)17--26(--34) x (5--10)10--16(--18). Bracteoles linear (S.A. 12) 1--2.5 mm long. Calyx (9--11)11--12(--13) mm; lower half of calyx tube glabrous inside; middle tooth 3--5 x 2--2.5 mm; mucro c. 1 mm. Corolla yellow or yellowish with purple pattern on lower lip and purple markings on upper lip; upper lip 6--7 mm, lower lip 11.5--14 mm, corolla tube 10--12 mm.  $2n=34$ .

Flowering time. April, May.

Distribution. Messenia, Lakonia.

*S. mollissima* Willd.

Willdenow 1805 p. 60.

*S. decumbens* Pers., Persoon 1806 p. 123. [B-Herb. Willd. No. 10879].

Orig. coll. Greece. Habitat in insula Corfu. (B-Herbar Willdenow no. 10876, holotype).

Flowering shoots, 20--35 cm. Indumentum of patent soft hairs and numerous sessile glands, stalked glands sparse, occurring on calyx teeth only. All leaves petiolate. Petiole of lower leaves 10--20 mm. Leaf lamina ovate to ovate--triangular (S.A. 39, 39--76); green; base truncate or cordate; apex acute; margin crenate or serrate; in lower leaves 12--21(--31) x 9--16(--22) mm, in middle 16--29(--37) x (10--15)15--27 mm, in upper 14--32(--35) x 8--20(--27) mm. Verticillasters 6--10(--12)-flowered, 2--6(--10) per inflorescence.

Floral leaves petiolate, uppermost sometimes subsessile; lamina of lowest floral leaves 10--25(--32) x 5--23(--25) mm. Bracteoles narrowly elliptic or linear (S.A. 3, 12), 7--12 mm long. Calyx length (7--8--10(--11) mm, lower half of calyx glabrous inside; middle tooth 3--4.5 x 1.5--2.5 mm; mucro 0.5--2 mm. Corolla white with a pale purplish pattern on lower lip, upper lip without markings; upper lip 5--7 mm; lower lip 10--14 mm; corolla tube 9--12 mm.  $2n=34$ .

Flowering time. May, June.

Distribution. Corfu.

Note. The nomenclatural problems with *S. mollissima* Willd. and *S. decumbens* Pers. were discussed by Ascherson (1872). Two specimens on which the descriptions were based are kept at the Willdenow Herbarium in Berlin. Willdenow described *S. mollissima* from Corfu in *Hortus berolinensis* (1805), sheet no. 10876 (this number was misprinted in Ascherson's article as 10867; the correct number is 10876 according to information from Berlin). Persoon based his description of *S. decumbens* on a plant of unknown origin, cultivated in Paris, sheet no. 10879, and published it in his *Synopsis plantarum* 2(1) (1806). Ascherson remarked that the two herbarium specimens differed somewhat in leaf shape and calyx size, the leaves of *S. decumbens* being smaller and markedly cordate at the base and the calyx teeth larger. He had received a plant from Corfu, collected by Schrader in 1872, which was intermediate between the two specimens and he concluded that the differences were a result of variability in the species. -- My own collections from Corfu (9 localities) also show a wide range of variability. -- Only photographs of the Willdenow Herbarium sheets nos. 10876 and 10879 were available for study but the two specimens seem to belong to the same species. Since the description of *S. mollissima* was published earlier (1805), the name *S. mollissima* Willd. has priority over *S. decumbens* Pers. (1806).

*S. tetragona* Boiss. et Heldr.

Boissier 1879 p. 736.

Orig. coll. In regione inferiori m. Dirphyis (Delphi hod.) Euboeae supra pagum Steni, ad rupes 9 aug 1858. De Heldreich plantae exciccatae e Graecia No 3468 (G-Boiss., lectotype).

Flowering shoots 20--35 cm with long internodes. Indumentum sparse with geniculate and patent hairs, sessile glands (sparse to moderate) and stalked glands (sparse). Petioles of lower leaves 7--15 mm. Middle leaves narrowly attenuate; upper leaves sessile. Leaf lamina narrowly obovate or narrowly elliptic (S.A. 46, 46--47; 2); green; base attenuate; apex acute; margin crenate or serrate; in lower leaves 13--20 x 5--8 mm, in middle 20--38 x 4--10 mm, in upper 8--18(--23) x 2--3 mm. Verticillasters 2--6-flowered, distant, 1--2(--3) per inflorescence. Floral leaves sessile, narrowly ovate or linear (S.A. 36, 37, 12), lamina 2--7 x 0.5--1.5 mm. Bracteoles narrowly elliptic (S.A. 1, 2), 2--5 mm long. Calyx 6--8 mm; lower half of calyx tube glabrous inside; calyx teeth emucronate, broadly or transversely broadly triangular (S.A. 78, 79), 1.5--2.5 x 2--2.5 mm. Corolla whitish with a reddish lilac pattern on lower lip, upper lip without markings; upper lip 6--7 mm, lower lip 12--14 mm; corolla tube 9--11 mm.  $2n=34$ .

Flowering time. May, June.

Distribution. Euboea, Northern Sporades (Yiora, Kyra Panagia).



## MORPHOLOGICAL VARIATION

Variation has probably been the main cause of the many taxonomic changes which have been made within the *swainsonii* complex. Morphological variation is indeed very striking within a population as well as among populations and also among different geographical areas. Therefore, it was essential

- (1) to study the populations in detail in the field, to search for a variation pattern by analysing the environment within populations and to look for correlations,
- (2) to find out if the intrapopulation variation may be due to influence of environment as well as to differences in genetic constitution,
- (3) to establish the variation by statistical methods and other quantitative and qualitative analyses,
- (4) to reveal discontinuities, to analyse the geographical picture and to find some guide-lines for taxonomic treatment, and
- (5) to put the findings into the perspective of present-day knowledge of one of the important fields of botany, morphological variation.

## FIELD OBSERVATIONS

All taxa of the *swainsonii* complex are outcrossing perennials. They are restricted to calcareous rocks and are therefore, as a consequence of topographic conditions, separated into local populations. The populations vary in size from much less than a hundred to several hundreds or even up to thousands. Most plants grow on exposed rocks in some soil, others in cracks and crevices or on ledges together with other plants which often provide partial or total shade. Sometimes the rock itself may shade the habitat. Occasionally they are

found along road cuts. Populations grow on cliffs facing all directions although eastern and southern aspects were commonest among localities visited. The altitude ranges from a few metres above sea level to about 1500 m.

Populations on cliffs near the sea are more exposed to strong winds than are localities further inland; on the other hand they probably benefit from the coastal dew. The winters are mild and wet and the summers hot and dry.

In general the climate is approximately the same for all localities; there are no steep climatic boundaries cutting through the distribution area.

But for the individual plant the microclimate is more important.

The field work therefore included a study of the micro-environment. The purpose was to relate intrapopulation variation to ecological conditions and thereby find out if it was habitat correlated. A number of populations were studied in the Delphi area, on the Isthmus of Korinth, on the Argolis Peninsula and in Attika.

It should be kept in mind that each environmental factor is elaborately interrelated with many others; nevertheless, during field work the complexity of the environment must be reduced to simple terms. Therefore exposure to sunlight and wind, soil moisture, amount of soil and height above sea level were chosen to represent the climatic, edaphic and altitudinal gradients. These factors were estimated together with plant size, leaf size, leaf shape and pubescence and number of flowers. The soil conditions were sometimes difficult to judge. Many plants seemingly grew directly out of the rock, where it was impossible to estimate moisture. But sufficient water and nourishment were obviously available as no wilting plants were found.

## Results

The wide range of morphological variation was indeed very puzzling, but even the crude method used in the field disclosed some general trends common to all populations.

Plants growing in the dry sunny exposed habitats were invariably shorter, had smaller leaves, and were more pubescent than plants in moister shady

sheltered habitats. The large leaves of the shade plants were thinner, more rounded and often chlorotic and the pubescence was less dense. The temperature was lower, the light intensity lower, and the soil usually richer and moister in the shaded crevices. In general flowering was earlier and more abundant in the dry open sites and more nutlets were produced there. This is advantageous, since it must be more difficult for the seedlings to become established on the 'bare' rock than in a shady place where soil was always available. On the other hand competition with other plants was greater in the shade. 'Sun' and 'shade' plants were never mixed in the same kind of microhabitat even where changes in the environment occurred abruptly. But between the extremes of sunny dry and shady moist places, there were transitional habitats with numerous morphologically intermediate forms. Figs. 11 and 7 A.

Since the altitude of most populations was less than a hundred metres (often considerably less), no intrapopulation change attributable to this factor could be noticed. However the Delphi and the Parnis populations ranged about 400-500m in altitude. At Delphi plant size decreased only slightly and pubescence seemingly increased somewhat along the gradient, but no such effect was found at Mt Parnis. Grazing animals were sometimes seen on the mountain slopes, but grazed *Stachys* plants were never found.

All taxa are outbreeding and self-compatible. Pollination was relatively localized in the field; bees and *Bombus* pollinated flowers on the same plant (geitonogamy) and flowers on neighbouring individuals (vicinism). The seeds were dispersed within a close range. As the nutlets do not have any dispersal mechanism they fall near the mother plant.

In summary, the morphological intrapopulation variation seemed to be continuous and the phenotypical modification appreciable. Could the variation be in part explained by plasticity? Are there genetic differences between plants occupying the various microhabitats? These questions required further investigation. Therefore the Delphi population was chosen for a detailed study. This is one of the populations on Parnassos which because of intrapopulation





Fig. 11. Variants of *ssp. swainsonii* from natural habitats at Delphi. -- A: sun plant. -- B. one of the intermediate forms (often with narrower leaves). -- C: shade plant (upper part).



variation has been the subject of taxonomic discussion (Bornmüller 1928). The population is located on cliff faces and slopes on the south side of Mt Parnassos and measures about 500 by 400 m. Changes in the micro-environment sometimes occur abruptly. Nutlets from plants in shady, exposed and intermediate microhabitats were collected for experimental purposes and brought to Lund together with living plants and dried material.

#### GREENHOUSE EXPERIMENTS

Morphological characters studied in the field revealed phenotypic differences. But since the phenotype is the result of effects of both environment and genotype, it was necessary to investigate the heritability of the differences. Of course transplantations among the different micro-environments would have been preferable, but practical difficulties prevented this. Instead seeds of plants from shady, exposed and intermediate habitats were used. Plants were raised in a greenhouse

- (a) under varied environmental conditions -- for study of plasticity,
- (b) under uniform conditions -- for testing of character inheritance and
- (c) some plants were cross-pollinated -- for estimation of results of gene exchange.

##### (a) Varied environment experiment

Plants were raised under varied environmental conditions. The purpose was to induce response to simulated environments, to estimate the response and to record its direction.

##### Material and methods

Seeds of randomly selected plants from shady, exposed, and intermediate microhabitats were sown in pots which were placed at random in four simulated environments. The three categories were termed shade plants, sun plants and intermediate plants according to habitat of origin. The categories are also

referred to as variants (Briggs and Walters 1969) and subpopulations. The intermediate plants were treated as one category even though they represented a mixture of differing ecological conditions. It is usually considered that differences in light intensity and soil moisture contribute to phenotypical variation. Therefore an experiment in which the plants were subjected to four different treatments was carried out. These consisted of combinations of light and soil moisture as follows:

A = high soil moisture + high light intensity

B = high soil moisture + low light intensity

C = low soil moisture + high light intensity

D = low soil moisture + low light intensity

For convenience these environments were termed:

A = moist sun

B = moist shade

C = dry sun

D = dry shade

Terms according to Clausen (1940).

Shade, sun and intermediate plants were raised in each of these environments. The high-moisture pots (A and B) received four times as much water as the low-moisture ones (C and D). During summer the daily amount was 60 - 80 ml and 15 - 20 ml respectively. Occasionally on extremely hot days, the dry sun plants (C) began to wilt and were therefore given additional water. The other groups received proportional quantities. Watering was reduced during the rest of the year but the proportions were kept constant.

As no special environment-controlling equipment was available, shade environment was improvised by placing the pots under the tables, where little or no sun reached the plants. For simulating sun environment, the pots were placed on the tables and no shading of any kind was provided. On cloudy days in spring and summer natural light was supplemented by artificial light.

The maximum and minimum temperatures occurred about two hours later in the

shade than in the sun environment. The highest daily temperature was always associated with the lowest humidity and the lowest temperature with the highest humidity. The daily fluctuations were greater in the sun environment than in the shade, for instance  $23^{\circ}$  and 49 % (averages) vs.  $13^{\circ}$  and 37 % in the sun (June - Aug. 1974) and  $18^{\circ}$  and 42 % vs.  $6^{\circ}$  and 28 % (Sept. - Nov. 1974). The highest temperature recorded in the sun was  $38^{\circ}$  and in the shade  $31^{\circ}$ .

Again it must be stressed that the environment is a complex of interacting factors. The variables used here, light intensity and soil moisture, are covariables in the complex and may not be the most important factors but they can be looked upon as 'indicators' of certain types of environmental combinations, where they interact with temperature, humidity, light quality, wind and so on.

Height, calyx length and leaf size were the most variable characters in the field. Even though two of them are vegetative they need attention in a search for a solution of the problem of intraspecific variation especially when phenotypic modification seems to be involved.

The following characters were measured: height, calyx length, length and width of the lowest floral leaf, the first, second and third leaf below the inflorescence on the main stem, number of flowers per verticillaster and number of days from sowing to flowering. Occasional plants had no flowers, then the third to the sixth leaves above ground were measured. The calyx was split between the two lower teeth, flattened and measured from the base to the tip of the middle tooth. Flowers of the same age in the lowest verticillasters were used. Instead of ratio or surface area, leaf length and width were kept separate, so that the changes could be followed individually. Observations in the field had suggested that the two leaf characters were not always proportional and therefore might be governed by different combinations of factors. The plants were kept for another year for continued observation. The same characters were measured and compared with those from the previous year (1974). Pollen fertility was also determined in 1974.

An extensive experiment was started, but owing to unfortunate circumstances

many seedlings died and the experiment had to be carried through with a greatly reduced number of plants (104).

A nonparametric test, Kruskal-Wallis one-way analysis of variance (Siegel 1956), was used to estimate the 'effect' among environments and among variants within each environment. Measurements from two seasons, 1974 and 1975, were also compared.

## Results

Tables 1 and 2 show the measurements from 1974.

Characters of the shade, sun and intermediate plants were compared within each of the four environments. The results of the Kruskal-Wallis one-way analysis of variance are presented in Table 3.

The dry shade, D environment, gave significant differences in leaf width between the three variants. The other environments showed mostly nonsignificance, only leaf length (2nd and 3rd leaves) in A, moist sun, and floral leaf width in C, dry sun differed significantly.

Also, the response of each variant to the four environments was estimated. Table 4. The shade plants differed significantly among the various treatments in 6 out of 10 characters, the sun plants in height only and the intermediate plants showed no significant differences between the four environments.



Table 1. Character measurements, mean values 1974. Height in cm; calyx length, leaf length and width in mm. Number of plants in parenthesis.

|                |      | Height | Calyx<br>length | Fl.leaf<br>l. x w. | 1st caul.leaf<br>l. x w. | 2nd caul.leaf<br>l. x w. | 3rd caul.leaf<br>l. x w. | No.of plants<br>with max.no.<br>of flowers<br>per vert. |   |   |       | No.of<br>days sow-<br>ing to<br>flowering |
|----------------|------|--------|-----------------|--------------------|--------------------------|--------------------------|--------------------------|---------------------------------------------------------|---|---|-------|-------------------------------------------|
|                |      |        |                 |                    |                          |                          |                          | 0                                                       | 2 | 4 | 6 fl. |                                           |
| A. moist sun   |      |        |                 |                    |                          |                          |                          |                                                         |   |   |       |                                           |
| Shade plants   | ( 8) | 21     | 9.4             | 22.7 x 11.5        | 21.5 x 10.6              | 19.1 x 10.2              | 16.8 x 8.8               | -                                                       | 6 | 1 | 1     | 165                                       |
| Sun plants     | (10) | 22     | 8.8             | 24.6 x 12.0        | 25.4 x 11.8              | 24.8 x 11.0              | 24.1 x 11.8              | -                                                       | 4 | 1 | 5     | 152                                       |
| Interm. plants | (11) | 21     | 9.1             | 23.0 x 11.2        | 23.8 x 11.1              | 23.2 x 11.0              | 20.9 x 10.3              | -                                                       | 7 | - | 4     | 152                                       |
| B. moist shade |      |        |                 |                    |                          |                          |                          |                                                         |   |   |       |                                           |
| Shade plants   | (10) | 31     | 9.3             | 24.2 x 11.7        | 24.6 x 12.6              | 25.9 x 13.4              | 23.8 x 11.4              | 4                                                       | 6 | - | -     | 163                                       |
| Sun plants     | (11) | 25     | 9.5             | 21.5 x 11.8        | 20.9 x 11.3              | 20.1 x 10.8              | 18.8 x 10.9              | 2                                                       | 7 | 1 | -     | 153                                       |
| Interm. plants | (10) | 26     | 9.8             | 22.7 x 11.7        | 25.0 x 12.8              | 25.8 x 12.4              | 24.2 x 12.9              | -                                                       | 6 | 4 | -     | 159                                       |
| C. dry sun     |      |        |                 |                    |                          |                          |                          |                                                         |   |   |       |                                           |
| Shade plants   | ( 9) | 19     | 8.1             | 23.0 x 12.9        | 22.5 x 12.5              | 22.6 x 12.4              | 20.5 x 11.6              | -                                                       | 5 | 2 | 2     | 157                                       |
| Sun plants     | (10) | 17     | 8.4             | 22.0 x 11.1        | 22.6 x 11.2              | 23.1 x 11.2              | 22.9 x 11.3              | -                                                       | 3 | 5 | 2     | 148                                       |
| Interm. plants | ( 3) | 15     | 9.0             | 21.7 x 10.0        | 23.7 x 11.5              | 25.3 x 12.2              | 26.0 x 8.5               | -                                                       | 2 | 1 | -     | 160                                       |
| D. dry shade   |      |        |                 |                    |                          |                          |                          |                                                         |   |   |       |                                           |
| Shade plants   | ( 9) | 25     | 9.1             | 21.9 x 12.4        | 24.1 x 13.3              | 26.1 x 13.9              | 25.9 x 13.5              | -                                                       | 7 | 2 | -     | 154                                       |
| Sun plants     | (10) | 30     | 9.1             | 20.0 x 9.8         | 20.7 x 10.4              | 22.0 x 10.8              | 22.3 x 10.9              | 5                                                       | 4 | 1 | -     | 153                                       |
| Interm. plants | ( 3) | 30     | 9.3             | 22.7 x 14.2        | 22.7 x 14.0              | 24.0 x 14.3              | 25.3 x 14.3              | -                                                       | 2 | 1 | -     | 152                                       |

Table 2. Character measurements, mean values 1974. Height in cm; calyx length, leaf length and width in mm. Number of plants in parenthesis.

|                     |      | Height | Calyx<br>length | Fl.leaf<br>l. x w. | 1st caul,leaf<br>l. x w. | 2nd caul,leaf<br>l. x w. | 3rd caul,leaf<br>l. x w. | No.of plants<br>with max.no.<br>of flowers<br>per vert. |   |   |       | No.of<br>days sow-<br>ing to<br>flowering |
|---------------------|------|--------|-----------------|--------------------|--------------------------|--------------------------|--------------------------|---------------------------------------------------------|---|---|-------|-------------------------------------------|
|                     |      |        |                 |                    |                          |                          |                          | 0                                                       | 2 | 4 | 6 fl. |                                           |
| Shade plants        |      |        |                 |                    |                          |                          |                          |                                                         |   |   |       |                                           |
| A moist sun         | ( 8) | 21     | 9.4             | 22.7 x 11.5        | 21.5 x 10.6              | 19.1 x 10.2              | 16.8 x 8.8               | -                                                       | 6 | 1 | 1     | 165                                       |
| B moist shade       | (10) | 31     | 9.3             | 24.2 x 11.7        | 24.6 x 12.6              | 25.9 x 13.4              | 23.8 x 11.4              | 4                                                       | 6 | - | -     | 163                                       |
| C dry sun           | ( 9) | 19     | 8.1             | 23.0 x 12.9        | 22.5 x 12.5              | 22.6 x 12.4              | 20.5 x 11.6              | -                                                       | 5 | 2 | 2     | 157                                       |
| D dry shade         | ( 9) | 25     | 9.1             | 21.9 x 12.4        | 24.1 x 13.3              | 26.1 x 13.9              | 25.9 x 13.5              | -                                                       | 7 | 2 | -     | 154                                       |
| Sun plants          |      |        |                 |                    |                          |                          |                          |                                                         |   |   |       |                                           |
| A moist sun         | (10) | 22     | 8.8             | 24.6 x 12.0        | 25.4 x 11.8              | 24.8 x 11.0              | 24.1 x 11.8              | -                                                       | 4 | 1 | 5     | 152                                       |
| B moist shade       | (11) | 25     | 9.5             | 21.5 x 11.8        | 20.9 x 11.3              | 20.1 x 10.8              | 18.8 x 10.9              | 2                                                       | 7 | 1 | -     | 153                                       |
| C dry sun           | (10) | 17     | 8.4             | 22.0 x 11.1        | 22.6 x 11.2              | 23.1 x 11.2              | 22.9 x 11.3              | -                                                       | 3 | 5 | 2     | 148                                       |
| D dry shade         | (10) | 30     | 9.1             | 20.0 x 9.8         | 20.7 x 10.4              | 22.0 x 10.8              | 22.3 x 10.9              | 5                                                       | 4 | 1 | -     | 153                                       |
| Intermediate plants |      |        |                 |                    |                          |                          |                          |                                                         |   |   |       |                                           |
| A moist sun         | (11) | 21     | 9.1             | 23.0 x 11.2        | 23.8 x 11.1              | 23.2 x 11.0              | 20.9 x 10.3              | -                                                       | 7 | - | 4     | 152                                       |
| B moist shade       | (10) | 26     | 9.8             | 22.7 x 11.7        | 25.0 x 12.8              | 25.8 x 12.4              | 24.2 x 12.9              | -                                                       | 6 | 4 | -     | 159                                       |
| C dry sun           | ( 3) | 16     | 9.0             | 21.7 x 10.0        | 23.7 x 11.5              | 25.3 x 12.2              | 26.0 x 8.5               | -                                                       | 2 | 1 | -     | 160                                       |
| D dry shade         | ( 3) | 30     | 9.3             | 22.7 x 14.2        | 22.7 x 14.0              | 24.0 x 14.3              | 25.3 x 14.3              | -                                                       | 2 | 1 | -     | 152                                       |

Table 3. Comparison among shade, sun and intermediate plants, within each of the four environments (1974). A, moist sun; B, moist shade; C, dry sun; D, dry shade. Kruskal-Wallis one-way analysis of variance, values of H. Sample sizes: A: 8, 10, 11; B: 10, 11, 10; C: 9, 10, 3; D: 9, 10, 3. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

|                    | A      | B    | C      | D        |
|--------------------|--------|------|--------|----------|
| Height             | 0.64   | 1.24 | 3.66   | 1.99     |
| Calyx length       | 1.78   | 0.51 | 4.18   | 0.32     |
| Floral leaf length | 1.07   | 1.10 | 0.59   | 2.68     |
| " " width          | 0.62   | 0.02 | 9.73** | 9.55**   |
| 1st leaf length    | 3.06   | 2.75 | 1.26   | 4.13     |
| " " width          | 0.90   | 1.66 | 3.59   | 12.33**  |
| 2nd leaf length    | 7.18*  | 4.55 | 1.65   | 6.10*    |
| " " width          | 1.11   | 3.90 | 2.45   | 13.88*** |
| 3rd leaf length    | 9.50** | 4.00 | 3.21   | 2.44     |
| " " width          | 5.83   | 1.72 | 1.85   | 6.92*    |

Table 4. The four environments compared within each variant (1974). (Kruskal-Wallis).

Sample sizes: shade plants,  $n = 8, 9, 8, 9$ ; sun plants,  $n = 10, 11, 10, 10$ ; intermediate plants,  $n = 11, 10, 3, 3$ .

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

|                    | Shade plants | Sun plants | Intermediate plants |
|--------------------|--------------|------------|---------------------|
| Height             | 9.19*        | 13.56**    | 7.39                |
| Calyx              | 10.74*       | 3.83       | 1.92                |
| Floral leaf length | 1.12         | 6.54       | 0.26                |
| " " width          | 2.40         | 6.50       | 7.02                |
| 1st leaf length    | 1.85         | 6.80       | 0.72                |
| " " width          | 7.02         | 2.64       | 6.03                |
| 2nd leaf length    | 10.16*       | 5.68       | 1.50                |
| " " width          | 10.57*       | 0.28       | 5.29                |
| 3rd leaf length    | 11.10*       | 3.60       | 3.78                |
| " " width          | 13.01**      | 1.03       | 6.69                |

Even though most differences were statistically nonsignificant, certain trends were revealed. (1974)

Height was the most clear-cut environmental indicator. All plants in sunny environments were definitely shorter than those in shady ones (shortest in C, dry sun). Sun and intermediate plants grew tallest in D, dry shade, while shade plants were largest in B, moist shade.

Calyx was longest in shady habitats (except for shade plants in A, moist sun).

Leaf size, each of the three variants responded differently:

Shade plants: All leaves were wider in D, and the second and third leaves were also longer than those in A and C.

Sun plants: The leaves were largest in A. Surprisingly leaves in B and D were smaller than in A and most leaves in C.

Intermediate plants: Leaves were ususally larger in B and D than in A and (sometimes) in C.

Number of flowers per verticillaster and number of days from sowing to flowering.

Table 1 shows that under simulated conditions the 6-flowered verticillasters occurred only in sun environments and plants without flowers only in shade. All plants exposed to sun also produced many more flowers and had a longer flowering period (especially marked in the sun plants).

The Kruskal-Wallis one-way analysis of variance of time from sowing to flowering gave significant differences among the variants in the A and the C environments. (Table 5).

Pollen stainability

The pollen stainability in 104 plants was: 99 plants -- > 80%, 4 plants -- 60-70%, 1 plant -- 30%.

Table 5. Time from sowing to flowering 1974.

Kruskal-Wallis one-way analysis of variance, values of H.

Among environments within each variant

df3; value of H significant at  $p < 0.05$ , 7.82.

| Shade plants | Sun plants | Intermediate plants |
|--------------|------------|---------------------|
| 7.25         | 0.76       | 2.15                |

Among variants within environments

df2; value of H significant at  $p < 0.05$ , 5.99

| A     | B    | C     | D    |
|-------|------|-------|------|
| 8.09* | 2.93 | 6.68* | 0.37 |

So far measurements from 1974 have been presented. The plants were kept during 1975 and the measurements repeated. The three variants were compared within each of the four environments and the results of the Kruskal-Wallis one-way analysis of variance are presented in Table 6.

In 1975 only two characters differed significantly, namely calyx length and floral leaves in the dry sun environment (C). In 1974 leaf width differed significantly in dry shade (D), but in 1975 though there were some differences they were nonsignificant.



Table 6. Shade, sun and intermediate plants compared within each environment 1974 and 1975. Kruskal-Wallis one-way analysis of variance, values of H. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

|                    | A         |      |             |      | B         |      |             |       | C        |         |           |          | D       |          |           |          |
|--------------------|-----------|------|-------------|------|-----------|------|-------------|-------|----------|---------|-----------|----------|---------|----------|-----------|----------|
|                    | moist sun |      | moist shade |      | moist sun |      | moist shade |       | dry sun  |         | dry shade |          | dry sun |          | dry shade |          |
|                    | 1974      | 1975 | 1974        | 1975 | 1974      | 1975 | 1974        | 1975  | 1974     | 1975    | 1974      | 1975     | 1974    | 1975     | 1974      | 1975     |
| Height             | 0.64      | 0.25 | 1.24        | 3.15 | 3.66      | 1.74 | 4.18        | 6.22* | 0.32     | 1.68    | 9.55**    | 2.46     | 4.13    | 1.61     | 12.33**   | 3.82     |
| Calyx length       | 1.78      | 3.26 | 0.51        | 0.36 | 0.59      | 2.56 | 0.02        | 1.13  | 9.73**   | 6.98*   | 1.26      | 3.38     | 3.59    | 5.48     | 6.10*     | 1.61     |
| Floral leaf length | 1.07      | 0.39 | 1.10        | 0.18 | 0.02      | 1.13 | 0.64        | 0.23  | 1.27     | 0.20    | 3.90      | 4.00     | 2.01    | 2.45     | 2.90      | 13.88*** |
| " " width          | 0.62      | 0.93 | 0.02        | 1.13 | 0.64      | 0.23 | 1.27        | 0.20  | 3.90     | 4.00    | 2.01      | 2.45     | 2.90    | 13.88*** | 2.44      | 1.61     |
| 1st leaf length    | 3.06      | 0.54 | 2.75        | 0.64 | 0.02      | 1.13 | 0.64        | 0.23  | 1.27     | 0.20    | 3.90      | 4.00     | 2.01    | 2.45     | 2.90      | 13.88*** |
| " " width          | 0.90      | 3.92 | 1.66        | 0.23 | 1.27      | 0.20 | 3.90        | 4.00  | 2.01     | 2.45    | 2.90      | 13.88*** | 2.44    | 1.61     | 12.33**   | 3.82     |
| 2nd leaf length    | 7.18*     | 1.58 | 4.55        | 1.27 | 1.65      | 2.81 | 2.45        | 2.90  | 13.88*** | 2.44    | 1.61      | 12.33**  | 3.82    | 6.10*    | 1.61      | 1.61     |
| " " width          | 1.11      | 5.58 | 3.90        | 0.20 | 2.45      | 2.90 | 13.88***    | 2.44  | 1.61     | 12.33** | 3.82      | 6.10*    | 1.61    | 1.61     | 1.61      | 1.61     |
| 3rd leaf length    | 9.50**    | 0.10 | 4.00        | 2.01 | 3.21      | 0.90 | 0.90        | 0.90  | 0.90     | 0.90    | 0.90      | 0.90     | 0.90    | 0.90     | 0.90      | 0.90     |
| " " width          | 5.83      | 5.53 | 1.72        | 0.24 | 1.85      | 1.33 | 1.85        | 1.33  | 1.85     | 1.33    | 1.85      | 1.33     | 1.85    | 1.33     | 1.85      | 1.33     |

Changes in character measurements from 1974 to 1975:

- A moist sun All variants: all characters, decrease -- in sun plants more than the others in leaf length and width.
- B moist shade All variants: height, marked increase; other characters, decrease -- most noticeable in shade plants.
- C dry sun All variants: height, slight increase; all other characters decrease -- less in shade plants than in sun and intermediate plants.
- D dry shade Shade and intermediate plants: height, marked increase.  
All variants: calyx measurements lacking (no flowers in 1975).  
Leaf characters, decrease -- except for 1st leaf length and width and 3rd leaf width in intermediate plants.

The Kruskal-Wallis test gave statistically significant and nonsignificant results which are indeed valuable. But behind these may lie important biological differences such as consistency of change. Thus a variant may show increase in one environment and decrease in another, or some characters on the same plant may show increase, others decrease in a consistent manner.

Logically each variant would be best adapted to its own natural habitat. A variant placed in a simulated environment resembling its natural habitat could be thought of as a 'standard', for instance sun plants in C (dry sun) and shade plants in B and D (moist shade, B is the commonest natural habitat for the shade plants). The intermediate plants come from varied habitats that are usually sunny and can therefore represent moist sun, A. If the standard environment is compared with the others, and increase and decrease recorded for each character, one could expect certain trends to appear. Each of the two seasons would have its own standard and therefore the effects of yearly fluctuations in the greenhouse conditions would be eliminated. Thus the trends for each year would be found. Then by comparing the two seasons one could show whether the trends remained constant. This was done and the results are given in Table 7.

Table 7. Trends in the response to the various environments. The "natural habitat" of each variant is set as a standard, the other environments are compared with it and increase or decrease recorded for each environment. Where two or three environmental codes occur after a sign, they are placed in descending order of magnitude of difference.

+ increase = same amount of change                    A moist sun                    C dry sun  
- decrease ( ) negligible change                    B moist shade                    D dry shade  
+ no change \* no flowers in D environment 1975

|                    | Height | Calyx length | Floral leaf |        | 1st leaf |          | 2nd leaf    |        | 3rd leaf |         |
|--------------------|--------|--------------|-------------|--------|----------|----------|-------------|--------|----------|---------|
|                    |        |              | length      | width  | length   | width    | length      | width  | length   | width   |
| Shade plants       |        |              |             |        |          |          |             |        |          |         |
| moist shade (B)    |        |              |             |        |          |          |             |        |          |         |
| 1974               | -DAC   | -CD +A       | -CAD        | -A +DC | -DCA     | -CA +D   | -CA +D      | -CA +D | -CA +D   | -A +CD  |
| 1975               | -DAC   | -CA          | -CDA        | -CDA   | -CDA     | -CDA     | -CDA        | -DCA   | -DCA     | -CA +D  |
| Dry shade (D)      |        |              |             |        |          |          |             |        |          |         |
| 1974               | -AC +B | -C +(B) (A)  | +ACB        | -AB +C | -CA +B   | -BCA     | -(B) CA     | -BCA   | -BCA     | -CBA    |
| 1975               | -CA +B | *            | -A+(C) B    | -A +CB | -A +CB   | -A+(C) B | -CA +B      | -CA +B | -CA +B   | -BCA    |
| Sun plants (C)     |        |              |             |        |          |          |             |        |          |         |
| 1974               | +ABD   | +ADB         | -DB +A      | -D +BA | -DB +A   | -D +BA   | -BD +A      | -AB=D  | -DB +A   | -B=D +A |
| 1975               | +ADB   | +AB *        | +ABD        | -A +DB | -A +DB   | -A +DB   | +<br>-A +DB | -A +DB | -A +DB   | -A +DB  |
| Interm. plants (A) |        |              |             |        |          |          |             |        |          |         |
| 1974               | -C +BD | -C +DB       | -B=DC       | -C +BD | -CD +B   | +CBD     | +DCB        | +CBD   | BDC      | -C +BD  |
| 1975               | -C +BD | +CB *        | +CDB        | +CBD   | +CBD     | +CBD     | +CDB        | +CBD   | +CBD     | + CBD   |

## Interpretation of Table 7.

'Standard' environment compared with the other three environments

Moist shade plants: B ('standard') compared with A, C and D.

1974 A: decrease (except calyx)

C: " (except floral leaf width and 3rd leaf width)

D: " (except all leaf widths and 2nd and 3rd leaf length)

1975 All environments: decrease (except 3rd leaf length in D)

Dry shade plants: D compared with A, B and C.

1974 A: decrease (except floral leaf length)

C: " (except floral leaf length and width)

B: " (except height, floral leaf length and first leaf length)

1975 A: decrease

C: " (except floral leaf width and first leaf length)

B: increase (except 3rd leaf width)

Sun plants: C compared with A, B and D.

1974 A: increase (except 2nd leaf width)

B: decrease (except height, calyx, floral leaf width, first leaf width)

D: " (except height, calyx)

1975 A: height, calyx and floral leaf length, slight increase; the rest of the characters, decrease

B: increase

D: "

Intermediate plants: A compared with B, C and D.

1974 B and D: increase

C: decrease (except 2nd and 3rd leaf length and 1st and 2nd leaf width)

1975 B: increase

D: marked increase

C: slight increase (except height)

## Conclusion

Even though the plants could not be expected to respond to the same extent in the simulated environments as under natural conditions a certain differential



response was noted. The shade plants were the most sensitive. The sun plants were 'slow' to respond to the shade environments, where they could be expected to increase in size. During the first season they increased in height and calyx length but not until the second season did the leaves become larger. The intermediate plants showed no statistically significant differences among the four environments but the differences in height and leaf width were pronounced. To sum up, the results of the experiment implied that the plants to some degree responded to environmental variables and expressed plasticity. Also, the fairly consistent pattern of change within each variant suggests differential performance, which in turn would indicate genetic differences.

#### (b) Transplant and progeny examinations

The results of the varied environment experiment pointed to possible genetic differences in the variants. The living material brought from Greece was therefore grown in a greenhouse for the purpose of finding out if the distinguishing features of each individual would be maintained in cultivation. Some indications of homogeneity of genotype would also be obtained by testing for character inheritance. Therefore, progeny of the transplants and of some selected individuals were raised from seed and grown under uniform conditions.

#### Transplants

The transplants originated from different types of microhabitat. They were kept in cultivation for several years and parts of the plants were dried each season and then compared with the field collections (portions of the transferred individuals had been dried in the field). These plants as well as all others kept in cultivation were placed at random in the greenhouse.

The plants often increased in height and leaf size, but leaf shape still conformed to that of each individual. The shade plants kept their large wide leaves, although usually not quite as large as in nature. The sun and intermediate plants sometimes developed relatively large leaves, but they were not

as wide as those of the shade plants; they kept their narrower shape and more cuneate base. Pubescence was less dense in the greenhouse material. The verticillasters were more remote in some long-stemmed plants. The number of flowers per verticillaster generally remained the same for each individual from year to year. Sun plants in particular flowered abundantly. Even though the variants tended to approach one another somewhat in cultivation the individual plants kept their distinguishing features.

### Progeny

The progeny of the transplants and some selected individuals were compared with the parent plants and with one another. The variation was most noticeable in height. Occasionally the habit varied; an ascending plant gave rise to ascending and decumbent types. A first-year plant with long decumbent branches and remote verticillasters often had a shorter ascending habit with denser inflorescences the following season. Calyx size varied, sometimes considerably. Greenhouse conditions probably accounted for much of this modification. Leaf shape usually remained constant.

Some of the progeny were self-pollinated. The descendents resembled the parents and no segregation or signs of inbreeding depressions appeared.

In summary, the progeny did not differ conspicuously from the parent plants, which suggests inheritance of characters. Also, genotypic differences among subpopulations are implied. Each variant seemed to be adapted to its original micro-environment; this is supported by observations of habitat correlations in the field.

### (c) Cross-pollination

The progeny study indicated that the Delphi population consists of different subpopulations associated with typical microhabitats. As the *S. swainsonii* complex is outbreeding and insect-pollinated it was of interest to investigate aspects of gene exchange and intrinsic isolation mechanisms between subpopulations.

No external reproductive isolation exists between them (seasonal, mechanical and ethological) and cross-pollination occurs among the variants. Internal reproductive isolation was investigated by artificial cross-pollination (for methods, see Crossing experiments).

Crosses between shade, sun and intermediate plants proved successful and the 'hybrids' developed normally. The first generation was usually intermediate in morphological characters. In some combinations individuals resembling the shade parent dominated. One sun x shade cross gave a plant with rather small rounded leaves, a form which occasionally occurs in the field. Another sun x shade combination resulted in an intermediate  $F_1$  family; the  $F_2$  plants were also very similar. Most of the  $F_1$  progeny had 2 (3, 4) flowers per verticillaster (sun parent usually 6, shade parent 2); sometimes 6-flowered verticillasters appeared in  $F_2$ . Combinations intermediate x shade plants yielded predominantly individuals with the narrower leaf shape.

Pollen stainability in  $F_1$  averaged 94 % (33 plants), in  $F_2$  93 % (10 plants). Seed set was very good in  $F_1$  and good in  $F_2$ . Chromosome counts in 14 plants gave  $2n = 34$ . Meiosis was normal in  $F_1$  of three combinations of crosses. Thus shade, sun and intermediate plants could be intercrossed with ease to yield fertile progeny. There were no apparent internal reproductive barriers between the variants.

## Conclusions

Trends in the experimental results suggested that the variation within the Delphi population was environmentally influenced as well as genetically determined. Evidence from field observations and cultivation analyses implied that the population consisted of several subpopulations, each adapted to its characteristic microhabitat within the population area. Each subpopulation seemed to have a certain range of plasticity which was genetically determined. No barriers to free gene exchange between the subpopulations seemed to exist.

Microhabitat correlated variation was also found in *ssp. argolica* and *spruneri* populations. Shade or even partially shaded habitats were rare in *ssp. scyronica* and *ssp. melangavica*. Few shade plants were found and the size differences were not as conspicuous as in the other taxa.

## STATISTICAL ANALYSES

So far intrapopulation variation has been considered. But there was also variation among populations and among geographical areas. It was necessary to get a clear picture of the variation pattern and therefore statistical methods were applied. Plants were collected by random sampling and character measurements analysed by analysis of variance (anova). The variability within the Argolis populations was computed by the coefficient of variation.

## Material and methods

A preliminary analysis revealed what sample size should be used (Sokal and Rohlf 1969) and which characters should be studied. Fifteen populations were randomly chosen from five critical geographical areas. See Fig. 12 for locations. Although *spruneri* is a separate species which can always be recognized by the narrow cauline leaves, triangular sessile floral leaves and mainly hispid hairs, it was included in the anova for comparison with the Argolis populations in particular. Two shoots from each of thirty plants were collected by random sampling in each population. Flowers at the same stage of development were used for measurements. The position and condition of the first pair of stamens determined the stage (just before the anthers turned outwards). Even though the calyx, for instance, had not yet reached its





Fig. 12. Localities where random collections were made.

|               |                  |                   |
|---------------|------------------|-------------------|
| 1 Gravia      | 6 Akr. Melangavi | 11 Driopi         |
| 2 Lidhorikion | 7 Korinthos      | 12 Methana        |
| 3 Delphi      | 8 Navplion       | 13 Néa Epidhavros |
| 4 Arakhova    | 9 Dhrepanon      | 14 Parnis         |
| 5 Megara      | 10 Stavropodion  | 15 Akraifnion     |

full size, this time was preferred because it is easy to pinpoint. Later, at calyx maximum, there would be no floral characters to help determine an exact stage. Precise timing was essential since the measurements were being compared.

The following characters were measured:

Corolla: -- corolla tube length, mm -- the distance from the base to the point where the upper and lower lip join. Fig. 13

-- length of upper lip, mm. Fig. 13

-- length of lower lip, mm Fig. 13

-- angle of the lower lip, measured in degrees in one flower per plant Fig. 13

Pistil: -- pistil length, mm

Calyx: -- calyx length, mm. The calyx was split between the two lower teeth, flattened and measured from the base to the tip of the middle tooth (mucro not included) Fig. 13

-- middle tooth length Fig. 13

-- middle tooth width Fig. 13

-- middle tooth mucro Fig. 13

Measured in ocular units

16.2 units = 1 mm

(x 0.062 = mm)

-- long hairs (more than 0.6 mm long)

-- short hairs (less than 0.6 mm)

-- stalked glands

Amounts measured in

units on an arbitrary scale

from 0-4

Leaves: -- long hairs (more than 0.6 mm)

-- short hairs (less than 0.6 mm)

Amounts measured in units

on an arbitrary scale from

0-4

The amounts of long and short hairs were determined separately on an area of the middle part of the leaf to the right of the midvein on the upper surface of the first leaf below the inflorescence.

Leaf size and shape are extremely variable in all taxa and cannot be used as distinguishing characters (except in *spruneri*). Although ssp. *scyronica*, for instance, always has small leaves (7--16) x 4--10 mm), the leaves of sun plants of the other taxa may be of the same size and shape.

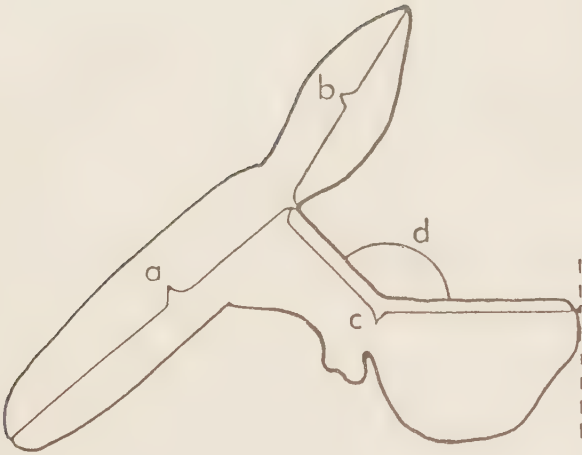
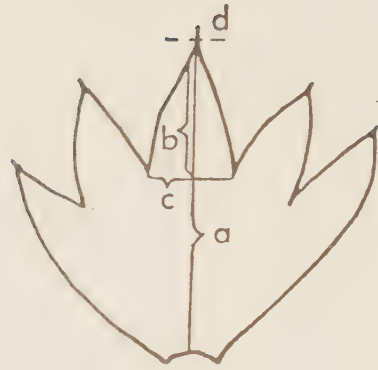


Fig. 13. Corolla measurements:

- a. corolla tube length
- b. upper lip length
- c. lower lip length
- d. angle of lower lip



Calyx measurements:

- a. calyx length
- b. middle tooth length
- c. middle tooth width
- d. mucro

## Analysis of variance (Anova)

Thirteen characters were analysed by hierarchic analysis of variance (Sokal & Rohlf 1969, Sokal 1962). Variance among populations, variance among individuals within populations, and variance within individuals were the three sources of variance, with 14, 435, and 450 degrees of freedom respectively. An additional character, angle of the lower lip was computed only among populations and among individuals within populations with 14 and 435 degrees of freedom. A desk calculator, Compucorp 445 Statistician, was used. The results are presented in Tables 8--21.

The first part of the anova table shows the preliminary overall anova. These data were analysed in greater detail (mixed model): comparisons had been designed to test among certain groups of populations -- tentative taxa -- and within these groups. Therefore the sum of squares and the degrees of freedom of the 'among populations' level from the preliminary anova were subdivided into a priori comparisons. These results are listed in the second part of the table.

Finally, a posteriori comparisons were made, using the SS - STP (Sum of squares simultaneous test procedure). The third part of the table summarizes the results. The population means are arrayed in ascending order of magnitude and the lines beside them represent nonsignificant sets of means. Thus, two means differ significantly at a probability level less than 0.01 if no line in the diagram includes both of them in its range. (Two means of the same value may occasionally differ in overlap pattern when their SS are close to the critical SS; this is due to the effect of rounding off.)



Tables 8--21. Analysis of variance. Measurements from 15 populations, 30 individuals per population and 2 flowers or 2 leaves per individual.

-- Anova table. Preliminary overall anova.

-- A priori comparisons. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

-- A posteriori comparisons. Populations are ranked in ascending order of magnitude of means. Any means connected by a line are not significantly different at  $P < 0.01$ .

Table 8. Anova table. Corolla tube length.

| Source of variation                  | df  | SS      | MS    | F         | A posteriori comparisons |      |  |
|--------------------------------------|-----|---------|-------|-----------|--------------------------|------|--|
| Among populations                    | 14  | 537.96  | 38.43 | 37.87***  | Pop. no.                 | Mean |  |
| Among individuals within populations | 435 | 441.39  | 1.01  | 4.10***   | 5                        | 8.2  |  |
| Within individuals                   | 450 | 111.43  | 0.25  |           | 6                        | 9.6  |  |
| Total                                | 899 | 1090.78 |       |           | 1                        | 9.7  |  |
|                                      |     |         |       |           | 2                        | 9.8  |  |
|                                      |     |         |       |           | 8                        | 9.8  |  |
|                                      |     |         |       |           | 9                        | 10   |  |
|                                      |     |         |       |           | 10                       | 10.3 |  |
|                                      |     |         |       |           | 3                        | 10.6 |  |
|                                      |     |         |       |           | 15                       | 10.6 |  |
|                                      |     |         |       |           | 12                       | 10.8 |  |
|                                      |     |         |       |           | 11                       | 10.8 |  |
|                                      |     |         |       |           | 4                        | 10.9 |  |
|                                      |     |         |       |           | 13                       | 11   |  |
|                                      |     |         |       |           | 14                       | 11   |  |
|                                      |     |         |       |           | 7                        | 11.5 |  |
| A priori comparisons                 |     |         |       |           |                          |      |  |
| Source of variation                  | df  | SS      | MS    | F         |                          |      |  |
| Among taxa                           | 4   | 359.99  | 90.00 | 144.07*** |                          |      |  |
| Among populations within taxa:       |     |         |       |           |                          |      |  |
| ssp. swainsonii                      | 3   | 60.78   | 20.26 | 32.43***  |                          |      |  |
| ssp. argolica                        | 6   | 114.02  | 19.00 | 30.42***  |                          |      |  |
| spruneri                             | 1   | 3.17    | 3.17  | 5.07*     |                          |      |  |
| Total                                | 14  | 537.96  |       |           |                          |      |  |

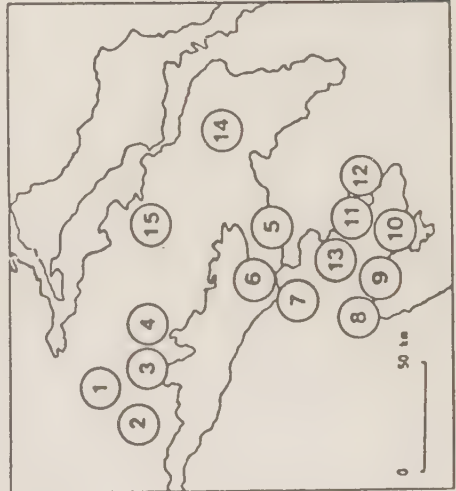


Table 9. Anova table. Length of upper lip.

| Source of variation                  | df  | SS     | MS    | F         | A posteriori comparisons |      |  |
|--------------------------------------|-----|--------|-------|-----------|--------------------------|------|--|
| Among populations                    | 14  | 311.22 | 22.23 | 35.86***  | Pop. no.                 | Mean |  |
| Among individuals within populations | 435 | 269.63 | 0.62  | 3.56***   | 5                        | 4.1  |  |
| Within individuals                   | 450 | 78.38  | 0.17  |           | 9                        | 4.8  |  |
| Total                                | 899 | 659.23 |       |           | 8                        | 4.9  |  |
|                                      |     |        |       |           | 10                       | 5.0  |  |
|                                      |     |        |       |           | 6                        | 5.0  |  |
|                                      |     |        |       |           | 12                       | 5.2  |  |
|                                      |     |        |       |           | 15                       | 5.2  |  |
|                                      |     |        |       |           | 11                       | 5.7  |  |
|                                      |     |        |       |           | 14                       | 5.7  |  |
|                                      |     |        |       |           | 1                        | 5.7  |  |
|                                      |     |        |       |           | 7                        | 5.8  |  |
|                                      |     |        |       |           | 2                        | 5.9  |  |
|                                      |     |        |       |           | 13                       | 5.9  |  |
|                                      |     |        |       |           | 3                        | 6.3  |  |
|                                      |     |        |       |           | 4                        | 6.3  |  |
| A priori comparisons                 |     |        |       |           |                          |      |  |
| Source of variation                  | df  | SS     | MS    | F         |                          |      |  |
| Among taxa                           | 4   | 207.74 | 51.94 | 132.07*** |                          |      |  |
| Among populations within taxa:       |     |        |       |           |                          |      |  |
| ssp. swainsonii                      | 3   | 15.54  | 5.18  | 13.17***  |                          |      |  |
| ssp. argolica                        | 6   | 82.20  | 13.70 | 34.04***  |                          |      |  |
| spruneri                             | 1   | 5.74   | 5.74  | 14.60***  |                          |      |  |
| Total                                | 14  | 311.22 |       |           |                          |      |  |

Table 10. Anova table. Length of lower lip.

| Source of variation  | df  | SS      | MS     | F         | A posteriori comparisons |      |  |
|----------------------|-----|---------|--------|-----------|--------------------------|------|--|
| Among populations    | 14  | 1060.22 | 75.73  | 54.35***  | Pop. no.                 | Mean |  |
| Among individuals    | 435 | 606.08  | 1.39   | 3.76***   | 5                        | 8.9  |  |
| Within individuals   | 450 | 166.66  | 0.37   |           | 6                        | 9.3  |  |
| Total                | 899 | 1832.96 |        |           | 8                        | 9.6  |  |
|                      |     |         |        |           | 9                        | 9.7  |  |
|                      |     |         |        |           | 10                       | 10.1 |  |
|                      |     |         |        |           | 15                       | 10.6 |  |
|                      |     |         |        |           | 12                       | 10.8 |  |
|                      |     |         |        |           | 14                       | 11.0 |  |
|                      |     |         |        |           | 11                       | 11.3 |  |
|                      |     |         |        |           | 13                       | 11.5 |  |
|                      |     |         |        |           | 7                        | 11.5 |  |
|                      |     |         |        |           | 2                        | 11.7 |  |
|                      |     |         |        |           | 1                        | 11.8 |  |
|                      |     |         |        |           | 3                        | 12.3 |  |
|                      |     |         |        |           | 4                        | 12.6 |  |
| A priori comparisons |     |         |        |           |                          |      |  |
| Source of variation  | df  | SS      | MS     | F         |                          |      |  |
| Among taxa           | 4   | 774.77  | 193.69 | 221.83*** |                          |      |  |
| Among populations    |     |         |        |           |                          |      |  |
| within taxa:         |     |         |        |           |                          |      |  |
| ssp. swainsonii      | 3   | 31.60   | 10.53  | 12.06***  |                          |      |  |
| ssp. argolica        | 6   | 249.35  | 41.56  | 47.60***  |                          |      |  |
| spruneri             | 1   | 4.50    | 4.50   | 5.16*     |                          |      |  |
| Total                | 14  | 1060.22 |        |           |                          |      |  |

Table 11. Anova table. Angle of the lower lip.

| Source of variation  | df  | SS       | MS       | F         | A posteriori comparisons |      |  |
|----------------------|-----|----------|----------|-----------|--------------------------|------|--|
| Among populations    | 14  | 63339.68 | 4524.26  | 68.33***  | Pop. no.                 | Mean |  |
| Among individuals    |     |          |          |           | 15                       | 126  |  |
| within populations   | 435 | 28803.60 | 66.22    |           | 14                       | 120  |  |
| Total                | 449 | 92143.28 |          |           | 10                       | 131  |  |
|                      |     |          |          |           | 13                       | 133  |  |
|                      |     |          |          |           | 7                        | 133  |  |
|                      |     |          |          |           | 9                        | 133  |  |
|                      |     |          |          |           | 11                       | 134  |  |
|                      |     |          |          |           | 2                        | 136  |  |
|                      |     |          |          |           | 8                        | 137  |  |
|                      |     |          |          |           | 3                        | 139  |  |
|                      |     |          |          |           | 12                       | 139  |  |
|                      |     |          |          |           | 1                        | 140  |  |
|                      |     |          |          |           | 4                        | 140  |  |
|                      |     |          |          |           | 6                        | 157  |  |
|                      |     |          |          |           | 5                        | 175  |  |
| A priori comparisons |     |          |          |           |                          |      |  |
| Source of variation  | df  | SS       | MS       | F         |                          |      |  |
| Among taxa           | 4   | 61624.13 | 15406.03 | 232.67*** |                          |      |  |
| Among populations    |     |          |          |           |                          |      |  |
| within taxa:         |     |          |          |           |                          |      |  |
| ssp. swainsonii      | 3   | 258.40   | 86.13    | 1.30 ns   |                          |      |  |
| ssp. argolica        | 6   | 1363.40  | 227.23   | 3.43**    |                          |      |  |
| spruneri             | 1   | 93.75    | 93.75    | 1.42 ns   |                          |      |  |
| Total                | 14  | 63339.68 |          |           |                          |      |  |

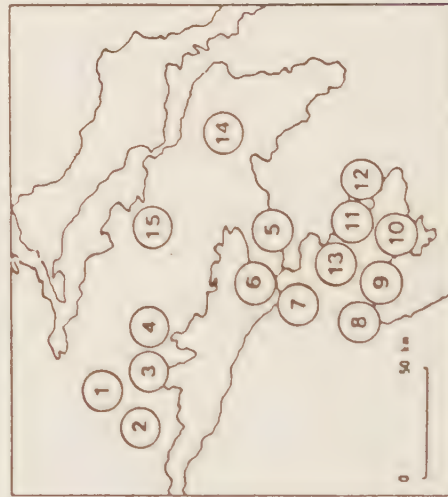


Table 12. Anova table. Pistil length.

| Source of variation                  | df  | SS        | MS     | F         | A posteriori comparisons |      |
|--------------------------------------|-----|-----------|--------|-----------|--------------------------|------|
| Among populations                    | 14  | 1616.47   | 115.46 | 75.28***  | Pop. no.                 | Mean |
| Among individuals within populations | 435 | 667.21    | 1.53   | 4.18***   | 5                        | 9.2  |
| Within individuals                   | 450 | 164.97    | 0.37   |           | 6                        | 11.5 |
| Total                                | 899 | 2448.65   |        |           | 8                        | 12.7 |
|                                      |     |           |        |           | 9                        | 13.1 |
|                                      |     |           |        |           | 1                        | 13.2 |
|                                      |     |           |        |           | 2                        | 13.4 |
|                                      |     |           |        |           | 4                        | 14.0 |
|                                      |     |           |        |           | 3                        | 14.0 |
|                                      |     |           |        |           | 10                       | 14.0 |
|                                      |     |           |        |           | 12                       | 14.1 |
|                                      |     |           |        |           | 7                        | 14.2 |
|                                      |     |           |        |           | 11                       | 14.2 |
|                                      |     |           |        |           | 13                       | 14.2 |
|                                      |     |           |        |           | 14                       | 14.4 |
|                                      |     |           |        |           | 15                       | 14.4 |
| A priori comparisons                 |     |           |        |           |                          |      |
| Source of variation                  | df  | SS        | MS     | F         |                          |      |
| Among taxa                           | 4   | 1449.82   | 362.46 | 385.46*** |                          |      |
| Among populations within taxa:       |     |           |        |           |                          |      |
| ssp. swainsonii                      | 3   | 29.73     | 9.91   | 10.54***  |                          |      |
| ssp. argolica                        | 6   | 136.92    | 22.92  | 24.27***  |                          |      |
| spruneri                             | 1   | 0.0005    | 0.0005 | 0.0005 ns |                          |      |
| Total                                | 14  | 1616.4705 |        |           |                          |      |

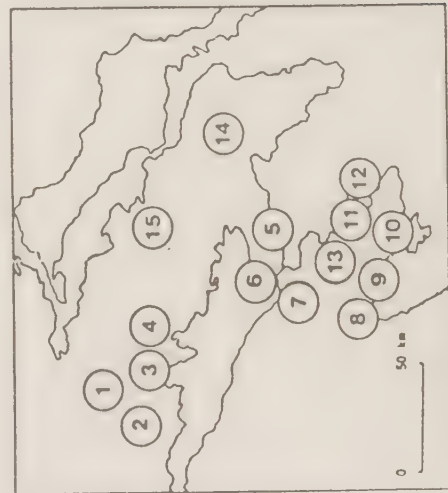


Table 13. Anova table. Calyx length.

| Source of variation                  | df  | SS      | MS    | F        | A posteriori comparisons |      |
|--------------------------------------|-----|---------|-------|----------|--------------------------|------|
| Among populations                    | 14  | 490.52  | 35.04 | 17.59*** | Pop. no.                 | Mean |
| Among individuals within populations | 435 | 866.47  | 1.99  | 7.02***  | 5                        | 6.7  |
| Within individuals                   | 450 | 127.72  | 0.28  |          | 12                       | 7.9  |
| Total                                | 889 | 1484.71 |       |          | 8                        | 8.3  |
|                                      |     |         |       |          | 6                        | 8.6  |
|                                      |     |         |       |          | 9                        | 8.7  |
|                                      |     |         |       |          | 11                       | 8.8  |
|                                      |     |         |       |          | 15                       | 8.9  |
|                                      |     |         |       |          | 1                        | 8.9  |
|                                      |     |         |       |          | 3                        | 9.0  |
|                                      |     |         |       |          | 4                        | 9.3  |
|                                      |     |         |       |          | 10                       | 9.3  |
|                                      |     |         |       |          | 14                       | 9.4  |
|                                      |     |         |       |          | 13                       | 9.4  |
|                                      |     |         |       |          | 2                        | 9.4  |
|                                      |     |         |       |          | 7                        | 9.9  |
| A priori comparisons                 |     |         |       |          |                          |      |
| Source of variation                  | df  | SS      | MS    | F        |                          |      |
| Among taxa                           | 4   | 310.52  | 77.63 | 69.20*** |                          |      |
| Among populations within taxa:       |     |         |       |          |                          |      |
| ssp. swainsonii                      | 3   | 10.53   | 3.51  | 3.12*    |                          |      |
| ssp. argolica                        | 6   | 163.17  | 27.20 | 24.21*** |                          |      |
| spruneri                             | 1   | 6.30    | 6.30  | 5.61*    |                          |      |
| Total                                | 14  | 490.52  |       |          |                          |      |



Table 14. Anova table. Calyx middle tooth length.

| Source of variation            | df  | SS       | MS      | F        | A posteriori comparisons |      |  |
|--------------------------------|-----|----------|---------|----------|--------------------------|------|--|
| Among populations              | 14  | 19178.43 | 1369.89 | 9.89***  | Pop. no.                 | Mean |  |
| Among individuals              |     |          |         |          | 5                        | 38.1 |  |
| Within populations             | 435 | 60255.42 | 138.52  | 8.53***  | 12                       | 42.8 |  |
| Within individuals             | 450 | 7305.50  | 16.23   |          | 8                        | 45.1 |  |
| Total                          | 889 | 86739.35 |         |          | 7                        | 45.1 |  |
|                                |     |          |         |          | 6                        | 46.1 |  |
|                                |     |          |         |          | 15                       | 49.0 |  |
|                                |     |          |         |          | 9                        | 49.6 |  |
|                                |     |          |         |          | 10                       | 50.0 |  |
|                                |     |          |         |          | 4                        | 50.0 |  |
|                                |     |          |         |          | 11                       | 50.4 |  |
|                                |     |          |         |          | 3                        | 51.7 |  |
|                                |     |          |         |          | 13                       | 52.0 |  |
|                                |     |          |         |          | 14                       | 52.6 |  |
|                                |     |          |         |          | 1                        | 53.1 |  |
|                                |     |          |         |          | 2                        | 57.8 |  |
| A priori comparisons           |     |          |         |          |                          |      |  |
| Source of variation            | df  | SS       | MS      | F        |                          |      |  |
| Among taxa                     | 4   | 12663.75 | 3165.94 | 41.47*** |                          |      |  |
| Among populations within taxa: |     |          |         |          |                          |      |  |
| ssp. swainsonii                | 3   | 2000.10  | 666.70  | 8.73***  |                          |      |  |
| ssp. argolica                  | 6   | 4140.05  | 690.01  | 9.04***  |                          |      |  |
| sprunerii                      | 1   | 374.58   | 374.58  | 4.91*    |                          |      |  |
| Total                          | 14  | 17173.43 |         |          |                          |      |  |



Table 15. Anova table. Calyx middle tooth width.

| Source of variation            | df  | SS       | MS     | F        | A posteriori comparisons |      |  |
|--------------------------------|-----|----------|--------|----------|--------------------------|------|--|
| Among populations              | 14  | 5217.35  | 372.67 | 15.04*** | Pop. no.                 | Mean |  |
| Among individuals              |     |          |        |          | 5                        | 24.7 |  |
| Within populations             | 435 | 10778.53 | 24.78  | 4.93***  | 6                        | 28.0 |  |
| Within individuals             | 450 | 2264.00  | 5.03   |          | 15                       | 29.8 |  |
| Total                          | 899 | 18259.88 |        |          | 2                        | 29.9 |  |
|                                |     |          |        |          | 3                        | 30.0 |  |
|                                |     |          |        |          | 14                       | 30.5 |  |
|                                |     |          |        |          | 1                        | 31.0 |  |
|                                |     |          |        |          | 9                        | 31.8 |  |
|                                |     |          |        |          | 12                       | 31.9 |  |
|                                |     |          |        |          | 3                        | 31.9 |  |
|                                |     |          |        |          | 4                        | 32.0 |  |
|                                |     |          |        |          | 8                        | 32.5 |  |
|                                |     |          |        |          | 10                       | 32.7 |  |
|                                |     |          |        |          | 13                       | 32.9 |  |
|                                |     |          |        |          | 11                       | 35.7 |  |
| A priori comparisons           |     |          |        |          |                          |      |  |
| Source of variation            | df  | SS       | MS     | F        |                          |      |  |
| Among taxa                     | 4   | 3959.00  | 989.75 | 67.16*** |                          |      |  |
| Among populations within taxa: |     |          |        |          |                          |      |  |
| ssp. swainsonii                | 3   | 171.83   | 57.28  | 3.89**   |                          |      |  |
| ssp. argolica                  | 6   | 1068.11  | 178.02 | 12.09*** |                          |      |  |
| sprunerii                      | 1   | 18.41    | 18.41  | 1.25 ns  |                          |      |  |
| Total                          | 14  | 5217.35  |        |          |                          |      |  |

Table 16. Anova table. Micro length

| Source of variation                  | df  | SS      | MS     | F        | A posteriori comparisons |      |  |
|--------------------------------------|-----|---------|--------|----------|--------------------------|------|--|
| Among populations                    | 14  | 1698.70 | 121.34 | 19.45*** | Pop. no.                 | Mean |  |
| Among individuals within populations | 435 | 2713.55 | 6.24   | 3.47***  | 13                       | 4.6  |  |
| Within individuals                   | 450 | 810.00  | 1.80   |          | 12                       | 4.9  |  |
| Total                                | 899 | 5222.25 |        |          | 4                        | 5.0  |  |
|                                      |     |         |        |          | 6                        | 5.3  |  |
|                                      |     |         |        |          | 11                       | 5.4  |  |
|                                      |     |         |        |          | 5                        | 5.4  |  |
|                                      |     |         |        |          | 3                        | 5.5  |  |
|                                      |     |         |        |          | 1                        | 5.7  |  |
|                                      |     |         |        |          | 7                        | 5.8  |  |
|                                      |     |         |        |          | 2                        | 6.3  |  |
|                                      |     |         |        |          | 10                       | 6.6  |  |
|                                      |     |         |        |          | 8                        | 7.1  |  |
|                                      |     |         |        |          | 9                        | 7.2  |  |
|                                      |     |         |        |          | 14                       | 7.9  |  |
|                                      |     |         |        |          | 15                       | 10.1 |  |
| A priori comparisons.                |     |         |        |          |                          |      |  |
| Source of variation                  | df  | SS      | MS     | F        |                          |      |  |
| Among taxa                           | 4   | 1121.31 | 280.33 | 70.41*** |                          |      |  |
| Among populations within taxa:       |     |         |        |          |                          |      |  |
| ssp. swainsonii                      | 3   | 54.04   | 18.02  | 4.52**   |                          |      |  |
| ssp. argolica                        | 6   | 378.15  | 63.02  | 15.83*** |                          |      |  |
| sprunerii                            | 1   | 145.20  | 145.20 | 36.47*** |                          |      |  |
| Total                                | 14  | 1698.70 |        |          |                          |      |  |

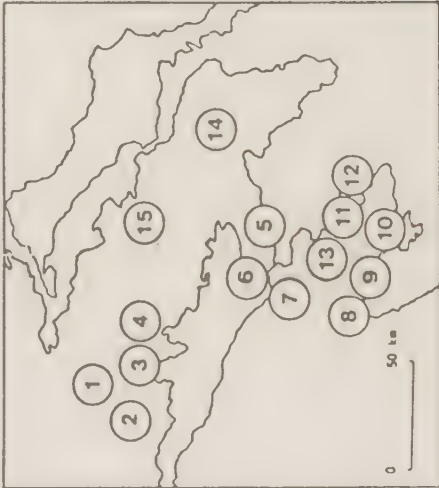


Table 17. Anova table. Calyx, long hairs.

| Source of variation.                 | df  | SS     | MS   | F        | A posteriori comparisons |      |  |
|--------------------------------------|-----|--------|------|----------|--------------------------|------|--|
| Among populations                    | 14  | 76.94  | 5.50 | 20.87*** | Pop. no.                 | Mean |  |
| Among individuals within populations | 435 | 114.58 | 0.26 | 3.25***  | 3                        | 2.3  |  |
| Within individuals                   | 450 | 36.50  | 0.08 |          | 11                       | 2.3  |  |
| Total                                | 889 | 228.02 |      |          | 2                        | 2.3  |  |
|                                      |     |        |      |          | 13                       | 2.3  |  |
|                                      |     |        |      |          | 9                        | 2.3  |  |
|                                      |     |        |      |          | 12                       | 2.4  |  |
|                                      |     |        |      |          | 5                        | 2.4  |  |
|                                      |     |        |      |          | 8                        | 2.4  |  |
|                                      |     |        |      |          | 4                        | 2.4  |  |
|                                      |     |        |      |          | 10                       | 2.6  |  |
|                                      |     |        |      |          | 14                       | 2.8  |  |
|                                      |     |        |      |          | 7                        | 2.9  |  |
|                                      |     |        |      |          | 15                       | 2.9  |  |
|                                      |     |        |      |          | 1                        | 3.0  |  |
|                                      |     |        |      |          | 6                        | 3.2  |  |
| A priori comparisons                 |     |        |      |          |                          |      |  |
| Source of variation                  | df  | SS     | MS   | F        |                          |      |  |
| Among taxa                           | 4   | 38.90  | 9.72 | 56.97*** |                          |      |  |
| Among populations within taxa:       |     |        |      |          |                          |      |  |
| ssp. swainsonii                      | 3   | 21.12  | 7.04 | 41.24*** |                          |      |  |
| ssp. argolica                        | 6   | 16.00  | 2.67 | 15.62*** |                          |      |  |
| sprunerii                            | 1   | 0.92   | 0.92 | 5.38*    |                          |      |  |
| Total                                | 14  | 76.94  |      |          |                          |      |  |

Table 18. Anova table. Calyx, short hairs.

| Source of variation                  | df  | SS     | MS    | F         | A posteriori comparisons |      |  |
|--------------------------------------|-----|--------|-------|-----------|--------------------------|------|--|
| Among populations                    | 14  | 291.87 | 20.85 | 68.96***  | Pop. no.                 | Mean |  |
| Among individuals within populations | 435 | 131.52 | 0.30  | 3.28***   | 14                       | 1.2  |  |
| Within individuals                   | 450 | 41.50  | 0.09  |           | 15                       | 1.5  |  |
| Total                                | 889 | 464.89 |       |           | 6                        | 1.7  |  |
|                                      |     |        |       |           | 11                       | 1.8  |  |
|                                      |     |        |       |           | 12                       | 1.8  |  |
|                                      |     |        |       |           | 9                        | 1.9  |  |
|                                      |     |        |       |           | 10                       | 2.0  |  |
|                                      |     |        |       |           | 8                        | 2.0  |  |
|                                      |     |        |       |           | 13                       | 2.1  |  |
|                                      |     |        |       |           | 2                        | 2.5  |  |
|                                      |     |        |       |           | 5                        | 2.7  |  |
|                                      |     |        |       |           | 3                        | 2.8  |  |
|                                      |     |        |       |           | 7                        | 2.8  |  |
|                                      |     |        |       |           | 1                        | 3.1  |  |
|                                      |     |        |       |           | 4                        | 3.2  |  |
| A priori comparisons                 |     |        |       |           |                          |      |  |
| Source of variation                  | df  | SS     | MS    | F         |                          |      |  |
| Among taxa                           | 4   | 233.91 | 58.48 | 299.12*** |                          |      |  |
| Among populations within taxa:       |     |        |       |           |                          |      |  |
| ssp. swainsonii                      | 3   | 17.50  | 5.83  | 29.85***  |                          |      |  |
| ssp. argolica                        | 6   | 37.61  | 6.27  | 32.06***  |                          |      |  |
| spruneri                             | 1   | 2.49   | 2.49  | 14.59***  |                          |      |  |
| Total                                | 14  | 291.87 |       |           |                          |      |  |

Table 19. Anova table. Stalked glands, calyx.

| Source of variation                  | df  | SS     | MS    | F         | A posteriori comparisons |      |  |
|--------------------------------------|-----|--------|-------|-----------|--------------------------|------|--|
| Among populations                    | 14  | 341.53 | 24.39 | 80.76***  | Pop. no.                 | Mean |  |
| Among individuals within populations | 435 | 131.41 | 0.30  | 4.18***   | 15                       | 0.1  |  |
| Within individuals                   | 450 | 32.53  | 0.07  |           | 14                       | 0.1  |  |
| Total                                | 899 | 505.47 |       |           | 7                        | 1.2  |  |
|                                      |     |        |       |           | 1                        | 1.4  |  |
|                                      |     |        |       |           | 6                        | 1.4  |  |
|                                      |     |        |       |           | 11                       | 1.6  |  |
|                                      |     |        |       |           | 13                       | 1.6  |  |
|                                      |     |        |       |           | 3                        | 1.7  |  |
|                                      |     |        |       |           | 12                       | 1.7  |  |
|                                      |     |        |       |           | 9                        | 1.9  |  |
|                                      |     |        |       |           | 4                        | 1.9  |  |
|                                      |     |        |       |           | 2                        | 1.9  |  |
|                                      |     |        |       |           | 8                        | 1.9  |  |
|                                      |     |        |       |           | 10                       | 2.1  |  |
|                                      |     |        |       |           | 5                        | 2.2  |  |
| A priori comparisons                 |     |        |       |           |                          |      |  |
| Source of variation                  | df  | SS     | MS    | F         |                          |      |  |
| Among taxa                           | 4   | 300.67 | 75.17 | 405.78*** |                          |      |  |
| Among populations within taxa:       |     |        |       |           |                          |      |  |
| ssp. swainsonii                      | 3   | 13.58  | 4.53  | 24.43***  |                          |      |  |
| ssp. argolica                        | 6   | 27.18  | 4.53  | 24.46***  |                          |      |  |
| spruneri                             | 1   | 0.10   | 0.10  | 0.55 ns   |                          |      |  |
| Total                                | 14  | 341.53 |       |           |                          |      |  |

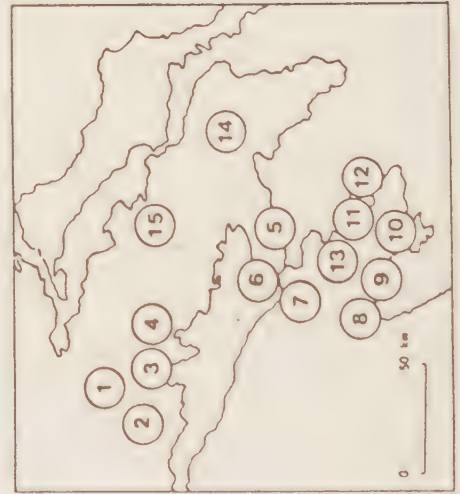


Table 20. Anova table. Long leaf hairs.

| Source of variation  | df  | SS     | MS    | F         | A posteriori comparisons |      |  |
|----------------------|-----|--------|-------|-----------|--------------------------|------|--|
| Among populations    | 14  | 454.83 | 32.49 | 56.49***  | Pop. no.                 | Mean |  |
| Among individuals    |     |        |       |           | 3                        | 0.8  |  |
| within populations   | 435 | 250.17 | 0.58  | 3.91      | 4                        | 1.0  |  |
| Within individuals   | 450 | 66.12  | 0.15  |           | 1                        | 1.1  |  |
| Total                | 899 | 771.12 |       |           | 2                        | 1.2  |  |
|                      |     |        |       |           | 8                        | 1.5  |  |
|                      |     |        |       |           | 9                        | 1.5  |  |
|                      |     |        |       |           | 14                       | 1.6  |  |
|                      |     |        |       |           | 13                       | 1.7  |  |
|                      |     |        |       |           | 12                       | 1.8  |  |
|                      |     |        |       |           | 15                       | 2.0  |  |
|                      |     |        |       |           | 7                        | 2.2  |  |
|                      |     |        |       |           | 11                       | 2.3  |  |
|                      |     |        |       |           | 10                       | 2.4  |  |
|                      |     |        |       |           | 6                        | 3.1  |  |
|                      |     |        |       |           | 5                        | 3.4  |  |
| A priori comparisons |     |        |       |           |                          |      |  |
| Source of variation  | df  | SS     | MS    | F         |                          |      |  |
| Among taxa           | 4   | 390.81 | 97.70 | 273.38*** |                          |      |  |
| Among populations    |     |        |       |           |                          |      |  |
| within taxa:         |     |        |       |           |                          |      |  |
| ssp. swainsonii      | 3   | 4.97   | 1.66  | 4.64**    |                          |      |  |
| ssp. argolica        | 6   | 54.25  | 9.04  | 25.30***  |                          |      |  |
| spruneri             | 1   | 4.80   | 4.80  | 13.43***  |                          |      |  |
| Total                | 14  | 454.83 |       |           |                          |      |  |

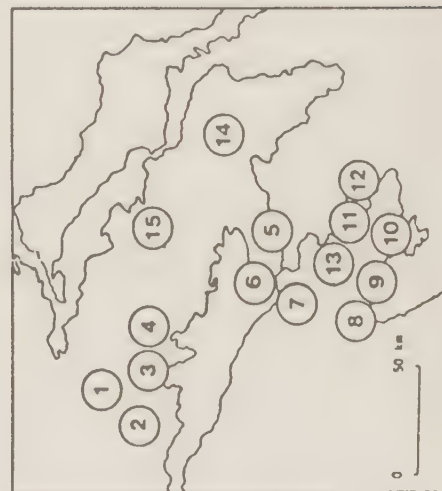


Table 21. Anova table. Short leaf hairs.

| Source of variation  | df  | SS     | MS    | F         | A posteriori comparisons |      |  |
|----------------------|-----|--------|-------|-----------|--------------------------|------|--|
| Among populations    | 14  | 286.74 | 20.48 | 34.44***  | Pop. no.                 | Mean |  |
| Among individuals    |     |        |       |           | 14                       | 1.1  |  |
| within populations   | 435 | 258.68 | 0.59  | 5.11***   | 15                       | 1.3  |  |
| Within individuals   | 450 | 52.40  | 0.12  |           | 12                       | 1.4  |  |
| Total                | 889 | 597.82 |       |           | 11                       | 1.6  |  |
|                      |     |        |       |           | 9                        | 1.6  |  |
|                      |     |        |       |           | 8                        | 1.7  |  |
|                      |     |        |       |           | 5                        | 1.8  |  |
|                      |     |        |       |           | 10                       | 1.9  |  |
|                      |     |        |       |           | 7                        | 1.9  |  |
|                      |     |        |       |           | 13                       | 2.0  |  |
|                      |     |        |       |           | 6                        | 2.1  |  |
|                      |     |        |       |           | 3                        | 2.7  |  |
|                      |     |        |       |           | 1                        | 2.7  |  |
|                      |     |        |       |           | 4                        | 2.9  |  |
|                      |     |        |       |           | 2                        | 2.9  |  |
| A priori comparisons |     |        |       |           |                          |      |  |
| Source of variation  | df  | SS     | MS    | F         |                          |      |  |
| Among taxa           | 4   | 269.87 | 67.47 | 191.94*** |                          |      |  |
| Among populations    |     |        |       |           |                          |      |  |
| within taxa:         |     |        |       |           |                          |      |  |
| ssp. swainsonii      | 3   | 2.87   | 0.96  | 2.72*     |                          |      |  |
| ssp. argolica        | 6   | 12.90  | 2.15  | 6.11***   |                          |      |  |
| spruneri             | 1   | 1.10   | 1.10  | 3.14 ns   |                          |      |  |
| Total                | 14  | 286.74 |       |           |                          |      |  |



## Results

### Anova

The anova tables show significant differences among populations for all characters analysed and also for 'among individuals within populations', except for long leaf hairs.

Populations were arranged in tentative taxa as follows: *ssp. swainsonii*, pops. 1-4 (Delphi area), *ssp. scyronica*, pop. 5 (Megara), *ssp. melangavica*, pop. 6 (Akr. Melangavi), *ssp. argolica*, pops. 7-13 (Argolis Peninsula), and *spruneri*, pops. 14-15, (Parnis and Akraifnion). When variation among populations within taxa was tested, the following picture emerged. At a probability level of less than 1 % *ssp. argolica* revealed significant differences among populations for all fourteen characters, *ssp. swainsonii* for eleven and *spruneri* for four characters.

*Ssp. scyronica*. The a posteriori comparisons disclosed considerable overlap. The Megara population (5), however, showed the lowest means seven times and the highest, three times and its mean values differed significantly from all others for five characters. Together with a few other special qualities, for instance the reddish-purplish marks on the upper lip, these results make the Megara plants distinct.

*Ssp. melangavica*. For six characters *ssp. melangavica* (6) either preceded or followed *ssp. scyronica*. Their means were significantly different for corolla tube length, pistil length and angle of the lower lip, nonsignificant for the other three. The total amount of pubescence was almost the same for both taxa, and their mean values for long leaf hairs were higher than those of all other populations (see also Fig. 19, p. 89). In percentages, Megara topped the list for long leaf hairs (66 %), followed by Akraifnion (60 %), and Akr. Melangavi, Driopi and Parnis (59 %).

*Sprunerii*. As a contrast the two *sprunerii* localities, Parnis (14) and Akraifnion (15), showed the lowest means for short hairs on both leaf and calyx and also for stalked calyx glands. The mucro was longer than in the other populations and sharpness seemed to increase proportionally. Furthermore the *sprunerii* flower had the most acute angle of the lower lip.

*Ssp. swainsonii*. Again, leaf pubescence proved to be distinguishing. The *ssp. swainsonii* populations (1-4) stand out on the a posteriori diagrams because their values for short leaf hairs were higher and for long leaf hairs lower than in other populations. All means for short calyx hairs and upper and lower lip were high even though there was considerable variation among the populations. Long calyx hairs was an extremely variable character. See also Fig. 19, p. 89.

*Ssp. argolica*. The a priori comparisons showed that *ssp. argolica* (7-13) was by far the most variable taxon. For easier interpretation a separate table of the seven Argolis populations was compiled (Table 22). The extreme means were always significantly different (angle of lower lip excepted) but overlapping made the variation continuous. There was only one discontinuity -- caused by the high mean value for short calyx hairs in the Korinthos population. However, this character was extremely variable at Korinthos. Otherwise short calyx hairs was the only character in which *ssp. argolica* as a taxon (excluding Korinthos) differed significantly from other taxa. It took a position between the low means of *sprunerii* and *ssp. melangavica* and the high means of *ssp. scyronica* and *ssp. swainsonii*. Unfortunately, this situation makes short calyx hairs a fairly poor field character because it might be difficult to estimate without having the other taxa at hand. See also p. 83.

The diagrams of the a posteriori comparisons can be thought of as a geographical interpretation of the variation expressed in abbreviated form. In Fig. 14 one of the diagrams (from Table 11, angle of lower lip) has been illustrated on a map. Localities (large circles) which are divided into 2 or 3 parts

show overlapping of as many lines.

As a summary, a posteriori comparisons among the five taxa are presented in Table 23, and illustrated on a geographical distribution map in Fig. 15.

Table 22. Results of a posteriori comparisons summarized for populations on the Argolis Peninsula (7-13).

| Corolla<br>tube<br>length | Upper lip | Lower lip | Angle | Pistil | Calyx<br>length | M. tooth<br>length |
|---------------------------|-----------|-----------|-------|--------|-----------------|--------------------|
| 8                         | 9         | 8         | 10    | 8      | 12              | 12                 |
| 9                         | 8         | 9         | 13    | 9      | 8               | 8                  |
| 10                        | 10        | 10        | 7     | 10     | 9               | 7                  |
| 12                        | 12        | 12        | 9     | 12     | 11              | 9                  |
| 11                        | 11        | 11        | 11    | 7      | 10              | 10                 |
| 13                        | 7         | 13        | 8     | 11     | 13              | 11                 |
| 7                         | 13        | 7         | 12    | 13     | 7               | 13                 |

| M. tooth<br>width | Mucro | Long<br>calyx<br>hairs | Short<br>calyx<br>hairs | Stalked<br>calyx<br>glands | Long<br>leaf<br>hairs | Short<br>leaf<br>hairs |
|-------------------|-------|------------------------|-------------------------|----------------------------|-----------------------|------------------------|
| 7                 | 13    | 11                     | 11                      | 7                          | 8                     | 12                     |
| 9                 | 12    | 13                     | 12                      | 11                         | 9                     | 11                     |
| 12                | 11    | 9                      | 9                       | 13                         | 13                    | 9                      |
| 8                 | 7     | 12                     | 10                      | 12                         | 12                    | 8                      |
| 13                | 10    | 8                      | 8                       | 9                          | 7                     | 10                     |
| 10                | 8     | 10                     | 13                      | 8                          | 11                    | 7                      |
| 11                | 9     | 7                      | 7                       | 10                         | 10                    | 13                     |

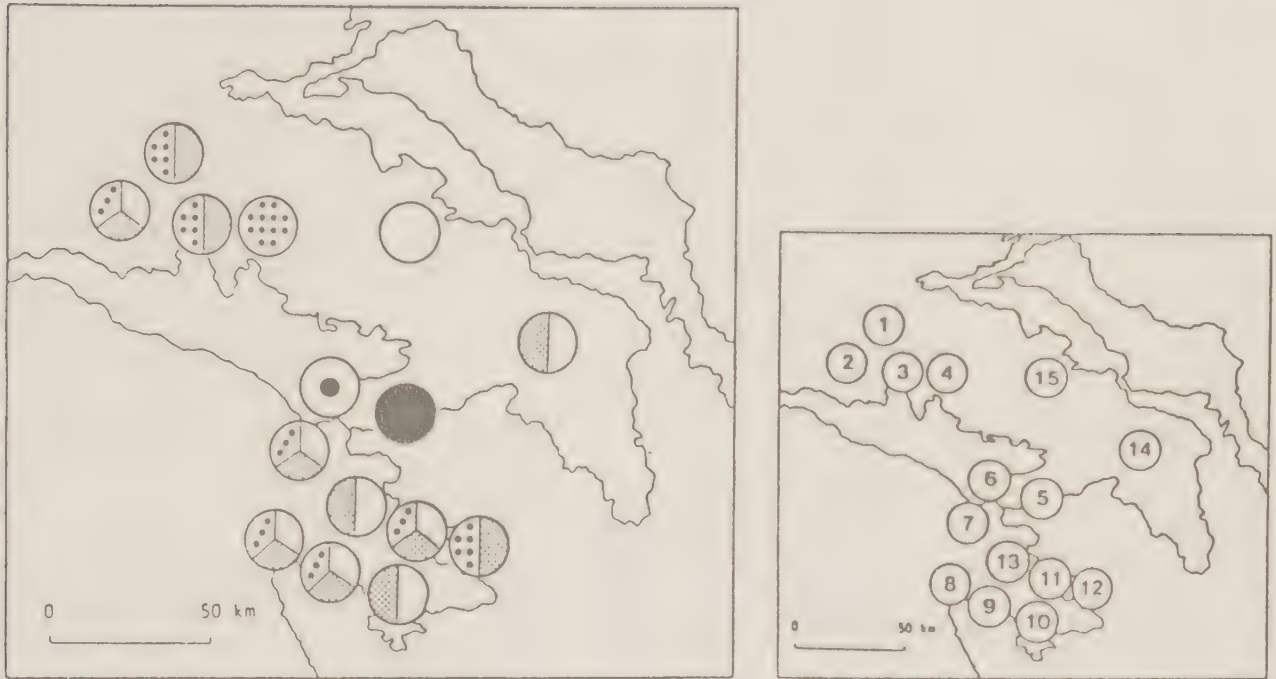


Fig. 14. Geographical variation according to the results of a posteriori comparisons for angle of lower lip (from Table 11). Groups of means, from lowest to highest, are represented by symbols: ○ ● ● ● ● .



Table 23. A posteriori comparisons. Populations arranged in taxa. Groups of means underlined (and bracketed where necessary) are not significantly different at  $P < 0.01$ .

|                                      | 1 - 4<br>ssp.<br>swain. | 5<br>ssp.<br>scyr. | 6<br>ssp.<br>mel. | 7 - 13<br>ssp.<br>arg. | 14 - 15<br>sprun |
|--------------------------------------|-------------------------|--------------------|-------------------|------------------------|------------------|
| Corolla tube length<br>mm            | 10.2                    | 8.2                | 9.6               | 10.6                   | 10.8             |
| Upper lip length<br>mm               | 6.0                     | 4.1                | 5.0               | 5.3                    | 5.5              |
| Lower lip length<br>mm               | 12.1                    | 8.9                | 9.3               | 10.6                   | 10.8             |
| Lower lip angle<br>degrees           | 139                     | 175                | 157               | 134                    | 127              |
| Pistil length<br>mm                  | 13.6                    | 9.2                | 11.5              | 13.8                   | 14.4             |
| Calyx length<br>mm                   | 9.1                     | 6.7                | 8.6               | 8.9                    | 9.1              |
| Middle tooth length<br>units         | 53                      | 38                 | 46                | 48                     | 51               |
| Middle tooth width<br>units          | 31                      | 25                 | 28                | 32                     | 30               |
| Mucro length<br>units                | 5.6                     | 5.4                | 5.3               | 5.9                    | 9                |
| Long calyx hairs<br>amount in units  | 2.5                     | 2.4                | 3.2               | 2.4                    | 2.8              |
| Short calyx hairs<br>amount in units | 2.9                     | 2.7                | 1.6               | 2.1                    | 1.3              |
| Stalked glands<br>amount in units    | 1.7                     | 2.2                | 1.4               | 1.7                    | 0.1              |
| Long leaf hairs<br>amount in units   | 1.0                     | 3.4                | 3.0               | 1.9                    | 1.8              |
| Short leaf hairs<br>amount in units  | 2.8                     | 1.8                | 2.1               | 1.8                    | 1.2              |

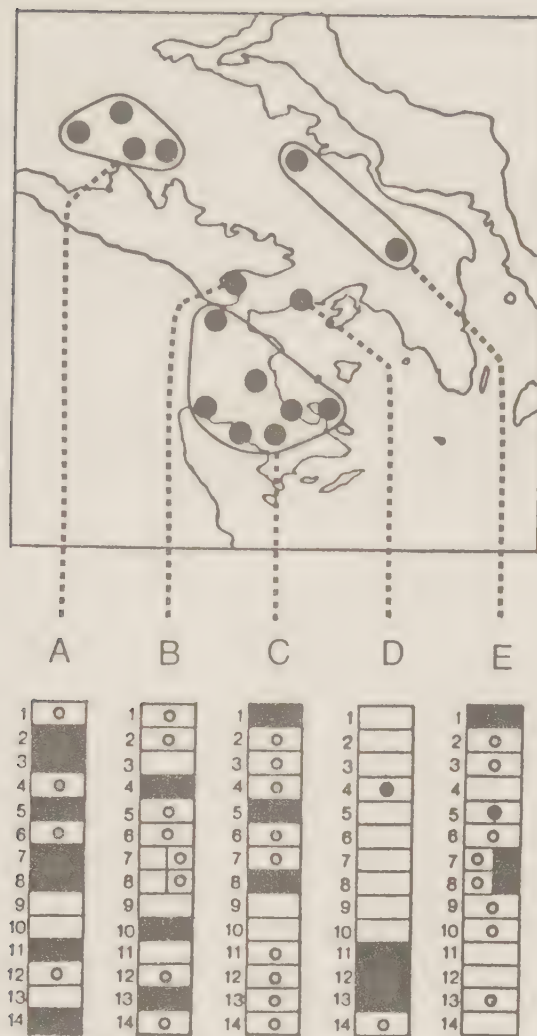


Fig. 15. Geographical distribution. Variation in the fourteen characters summarized in the five taxa.

□ ○ ■ = increasing order of magnitude of means.

Where four symbols are needed □● is added as the highest mean.

A: ssp. swainsonii. -- B: ssp. melangavica. -- C: ssp. argolica. -- D: ssp. scyronica. -- E: S. spruneri.

#### Characters:

- |                       |                       |                           |
|-----------------------|-----------------------|---------------------------|
| 1 corolla tube length | 6 calyx length        | 11 short calyx hairs      |
| 2 upper lip length    | 7 middle tooth length | 12 stalked glands (calyx) |
| 3 lower lip length    | 8 middle tooth width  | 13 long leaf hairs        |
| 4 lower lip angle     | 9 mucro               | 14 short leaf hairs       |
| 5 pistil length       | 10 long calyx hairs   |                           |

For an overall view of how the variation was distributed over the three sources, the variance components were calculated and expressed as percentages of the sum of their variances. Table 24. The results indicate that calyx characters (except short hairs and stalked glands) varied most among individuals. Mucro and long calyx hairs had similar percentages at all three levels. These two characters plus middle tooth width showed considerably greater 'within individual' variation than did others.

By contrast, corolla, leaf and pistil characters and also short calyx hairs and stalked glands definitely showed the highest percentages among populations.

Table 24. Percentages of the total variation attributable to each level of variation.

|         |                     | Among<br>popu-<br>lations | Among individ-<br>uals within<br>populations | Within individ-<br>uals |
|---------|---------------------|---------------------------|----------------------------------------------|-------------------------|
| Corolla | tube length         | 49.7                      | 30.3                                         | 20.0                    |
|         | upper lip           | 48.0                      | 29.3                                         | 22.7                    |
|         | lower lip           | 58.5                      | 24.1                                         | 17.4                    |
| Pistil  | length              | 66.7                      | 20.3                                         | 13.0                    |
| Calyx   | length              | 32.7                      | 50.6                                         | 16.7                    |
|         | middle tooth length | 21.0                      | 62.4                                         | 16.6                    |
|         | middle tooth width  | 24.3                      | 47.7                                         | 28.0                    |
|         | middle tooth mucro  | 32.3                      | 37.4                                         | 30.3                    |
|         | long hairs          | 34.0                      | 35.0                                         | 31.0                    |
|         | short hairs         | 63.0                      | 20.4                                         | 16.6                    |
|         | stalked glands      | 69.0                      | 19.0                                         | 12.0                    |
|         |                     |                           |                                              |                         |
| Leaf    | long hairs          | 59.5                      | 23.6                                         | 16.9                    |
|         | short hairs         | 47.8                      | 34.8                                         | 17.4                    |

## Coefficient of variation (CV %)

The variability of all characters was estimated by the coefficient of variation, CV %, (Sokal & Rohlf 1969) within each of the seven Argolis populations. By comparing the coefficients for a given character one can easily find out which population varies more than the others. Table 25.

Table 25. Coefficient of variation (%) for the seven Argolis populations.

| population number | corolla tube length | upper lip length | lower lip length | angle | pistil | calyx length | cal. mid. tooth length | cal. mid. tooth width | macro | long calyx hairs | short calyx hairs | stalked calyx glands | long leaf hairs | short leaf hairs |
|-------------------|---------------------|------------------|------------------|-------|--------|--------------|------------------------|-----------------------|-------|------------------|-------------------|----------------------|-----------------|------------------|
| 7                 | 8.7                 | 10.1             | 10.4             | 6.2   | 6.3    | 9.8          | 12.4                   | 11.6                  | 30.0  | 11.4             | 22.1              | 29.2                 | 24.9            | 20.0             |
| 8                 | 7.2                 | 12.4             | 9.8              | 5.7   | 6.9    | 10.7         | 19.2                   | 11.4                  | 32.6  | 17.9             | 21.0              | 21.6                 | 33.3            | 18.8             |
| 9                 | 5.4                 | 9.2              | 5.3              | 5.3   | 11.1   | 11.0         | 19.3                   | 9.2                   | 34.2  | 17.8             | 17.4              | 20.0                 | 31.6            | 29.4             |
| 10                | 7.0                 | 9.8              | 7.9              | 4.6   | 5.5    | 14.4         | 23.4                   | 10.8                  | 35.7  | 11.9             | 13.0              | 12.9                 | 23.8            | 24.3             |
| 11                | 8.1                 | 9.7              | 8.0              | 6.5   | 7.7    | 8.5          | 12.6                   | 8.5                   | 30.6  | 22.2             | 18.9              | 21.2                 | 24.1            | 29.8             |
| 12                | 7.1                 | 9.7              | 7.9              | 5.7   | 5.9    | 11.8         | 19.0                   | 11.2                  | 23.4  | 16.7             | 18.9              | 20.0                 | 28.3            | 23.2             |
| 13                | 6.3                 | 8.5              | 6.7              | 5.4   | 5.4    | 17.4         | 24.6                   | 13.8                  | 22.9  | 18.3             | 19.5              | 27.7                 | 31.1            | 27.7             |

When all characters are considered Korinthos and Navplion were the most variable populations, Methana and Stavropodion the least variable.



# ADDITIONAL CHARACTERS EXAMINED IN THE RANDOMLY COLLECTED MATERIAL

Number of teeth were counted on one side of each of four leaves: the second and third leaves below the inflorescence and the floral leaves of the two lowest verticillasters. The results are illustrated in Fig. 16. The overall averages for ssp. *argolica* almost equalled those for ssp. *swainsonii* but plants of population 10, 11, 12 and 13 usually had more teeth than those of populations 7, 8 and 9. The teeth were fewer in ssp. *scyronica* and ssp. *melangavica* which had the same averages except in the 2nd floral leaf where ssp. *melangavica* had one tooth less and sometimes also shallower teeth. The floral leaves of *spruneri* had the fewest and the shallowest teeth.

Bracteole length varied as follows: ssp. *swainsoni* 0-3.5 mm, ssp. *scyronica* 0-4 mm, ssp. *melangavica* 0-2.5 mm, ssp. *argolica* 0-11 mm and *spruneri* 0-5.5 mm. Plants with long bracteoles were commoner at Driopi and Methana (pops. 11 and 12, also N  a Epidhavros, 13, and Dhrepanon, 9) than in the other Argolis populations.

Ratio calyx length/corolla tube length has often been used to distinguish between related taxonomic entities in *Stachys*. Unfortunately, in spite of a certain correlation between the two characters, the ratio varied so much and the averages for the taxa were so close (0.82 - 0.93) that this ratio was of no taxonomic value.

Colours. Flower colours were estimated in the field in the randomly collected material. All populations have a basically white corolla with a coloured pattern on the lower lip. Colours of pattern, anthers and filaments were compared with colour charts in Kornerup & Wanscher (1974).

The colours matched those of charts number 12 and 13, bluish red to dark ruby, and purplish red to dark magenta. A few colours outside this range were noted,

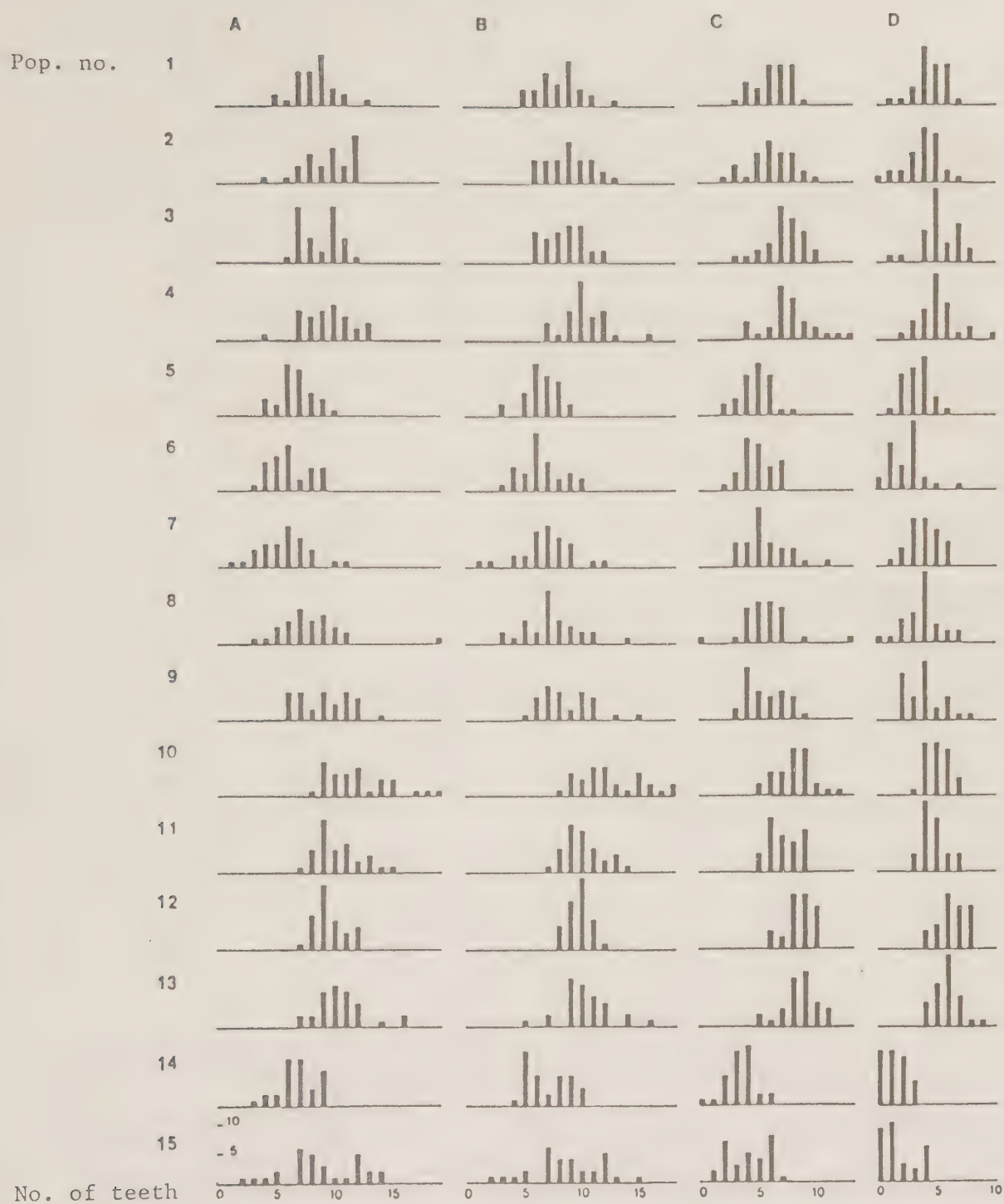


Fig. 16. Number of teeth. A: 3rd leaf below inflorescence. -- B: 2nd leaf below inflorescence. -- C: 1st floral leaf. -- D: 2nd floral leaf. -- 30 plants per population.

14 B4 and 5, reddish lilac on the pattern of the some of *Methana* flowers (pop. no. 12), and 2A2 and 4A2 and 4A3, yellowish white colours of some *swainsonii* and *argolica* anthers also the outer part of some *scyronica* anthers. As the pattern spreads over the lip, the colour often fades; therefore it was consistently measured at its greatest intensity. Fig. 17 shows the distribution of colours of patterns, anthers and filaments in each taxon. The revelant charts for each character have been placed in succession. The distribution of colours (%) for each taxon has been superimposed on the charts.

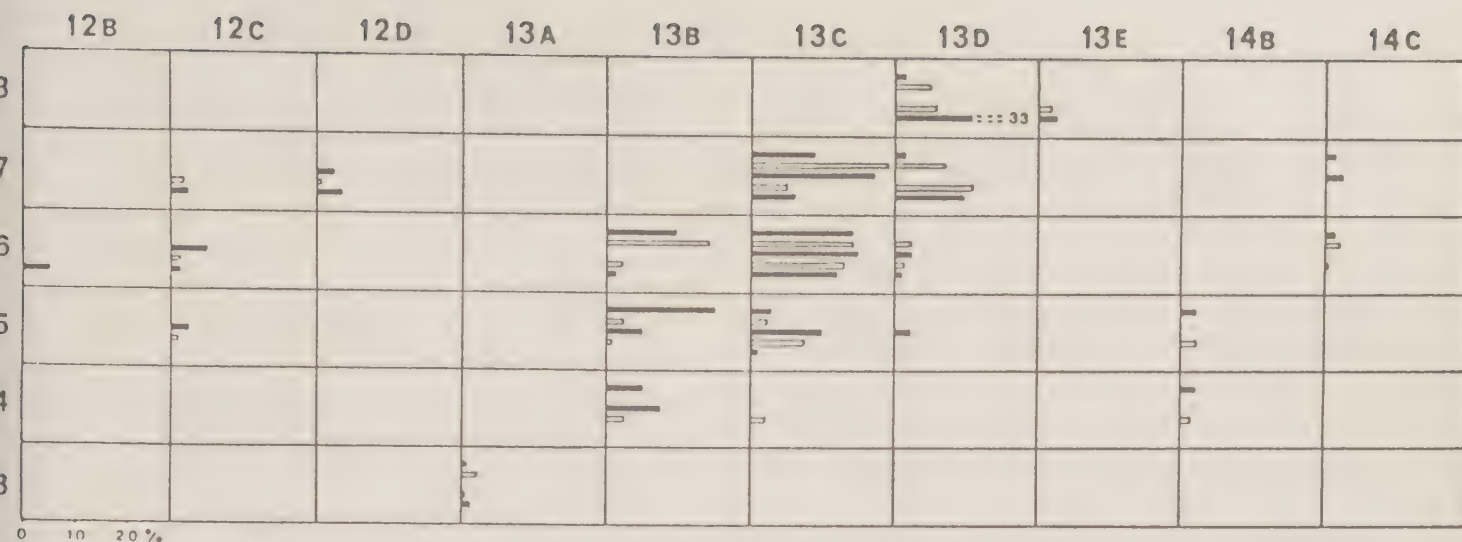
Almost all lower lip colours corresponded to those on chart 13. Flower pattern of *ssp. swainsonii* was usually paler than in other taxa, ranging in the B and C columns; *ssp. melangavica* and *ssp. scyronica* were mainly found in C and *ssp. argolica* and *spruneri* in C and D.

The lower lip pattern displayed an array of designs. Some definite lines and dots always point toward the corolla tube entrance (nectar guides). The pattern spreads over the lower part of the lip in innumerable arrangements of streaks and spots or occasionally as a solid, pale rose colour. Flowers without design were rarely found. Pattern on the upper lip occurs only in *ssp. scyronica*; it consists of marking along the margin about halfway up each side of the lip (deep magenta, 13D8, 13E8).

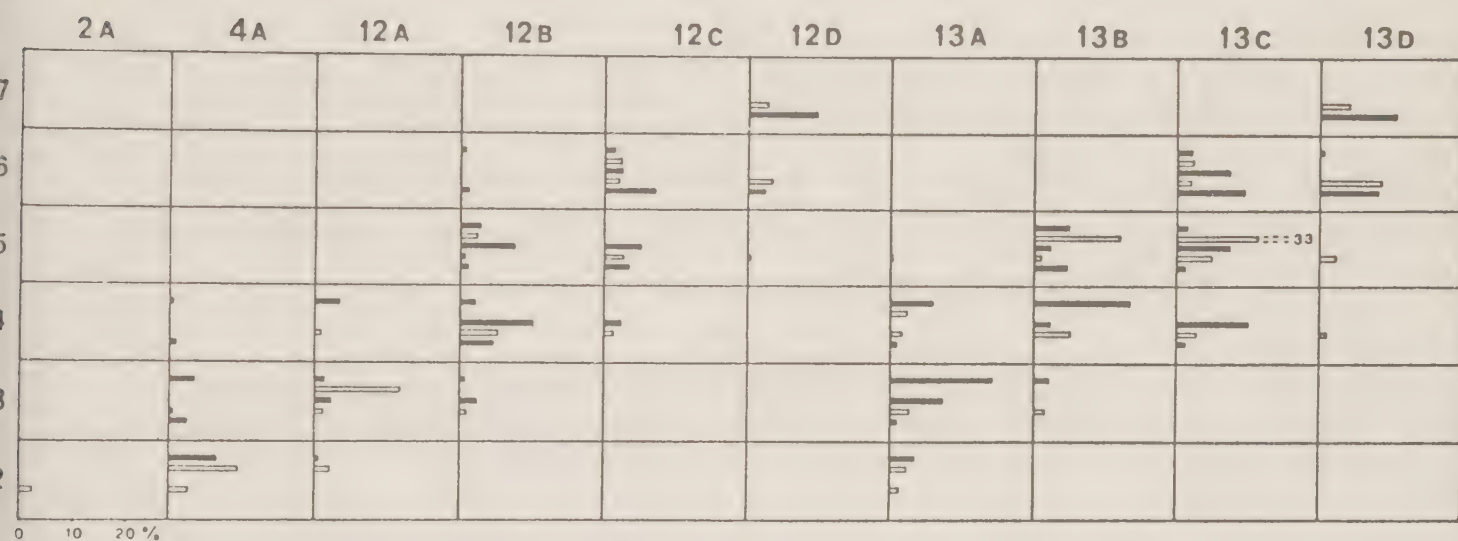
Unfortunately, intrapopulation colour variation of the lower lip as well as that of anthers and filaments was so great that colour alone cannot be used for taxonomic purposes.

Exsertion of the pistil means the distance from the lower fork of the stigma to the point where the upper and lower lips join. This character was measured in side view in flowers where the second pair of stamens had reached maturity. The reason for choosing a later stage of development here was that the interpopulation differences were more pronounced than earlier. Later as the pistil

A



B



C

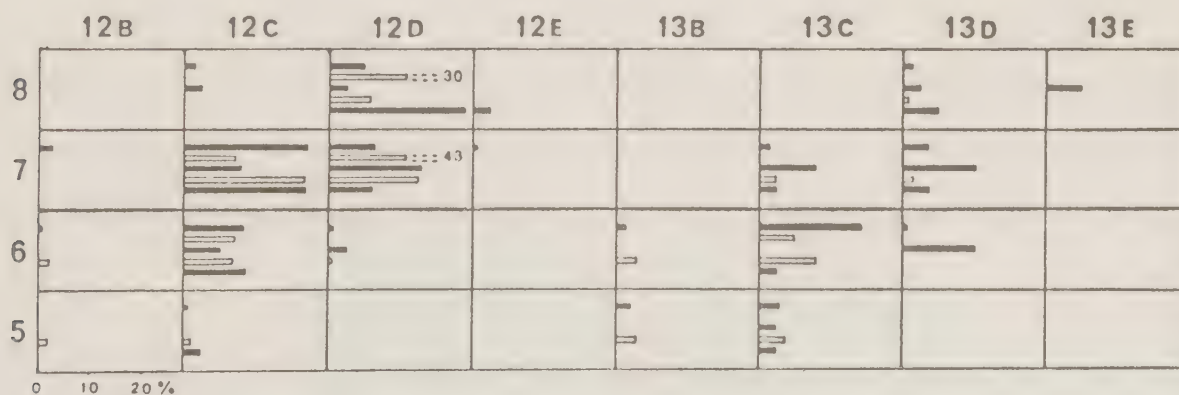


Fig. 17. Distribution of colours (%) of pattern of lower lip (A), anthers (B) and filaments (C) in each taxon. Colour designations after Kornerup and Wanscher (1974).

Each taxon has its own set position within a rectangle:

ssp. swainsonii —  
 ssp. scyronica —  
 ssp. melangavica —  
 ssp. argolica —  
 spruneri —



length increased, the differences became greater but then it was very difficult to determine an exact point in time. Fig. 18 shows that *ssp. scyronica* and *ssp. melangavica* (pops. 5 and 6) had much shorter pistils than the other taxa did.

Pollen stainability. Morphologically well-developed and well-stained pollen (lactophenol-aniline blue) were counted in the randomly collected plants. Some low percentages were found particularly in the Argolis populations. Since the mounts were prepared in the field one may have to allow for a greater error due to environmental factors than if they had been made in the greenhouse.

Table 26.

Age. Growth rings were counted in transverse sections of stems of plants from the Delphi area, Megara, Akr. Melangavi, Korinthos, Navplion, Dhrepanon and Parnis. The age ranged from 3 to 15 years (one plant 19 years) but older plants are likely to be found in the field.

Odour. All plants have a slight to moderately strong odour which is sometimes characteristic of a taxon (e.g. *euboica* and *ssp. scyronica*).

Some characters which were hardly noticeable or even lost in herbarium material attracted attention in the field. They pertained to floral architecture and dynamics of development (expansion of the lower lip, angle of the lower lip, exsertion of the pistil), colours and texture of foliage and colour changes of floral parts associated with age. Some of these features have already been discussed.

Expansion of the lower lip. The lower lip is  $\pm$  folded lengthwise. The angle formed by the sides varies, but is difficult to measure. Some changes also occur with age. Estimations showed considerable variability within local areas, but certain general trends were obvious: in *ssp. swainsonii* and *ssp. melangavica* the lower lip was flatter than in *ssp. argolica* and *spruneri*,

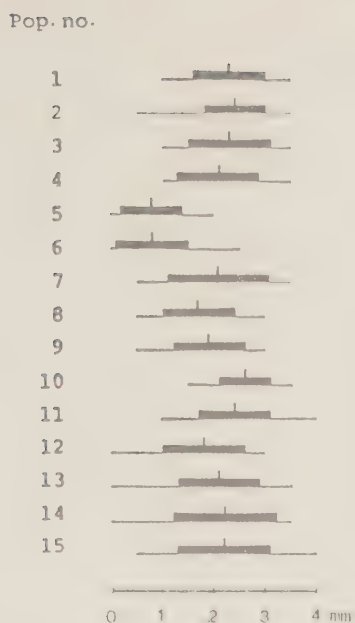


Fig. 18. Exsertion of the pistil, mm. The vertical line indicates the mean, the horizontal line the range, and the bar one standard deviation on each side of the mean. 30 plants per population.

28 30 23 25 66 59 54 46 48 56 59 56 46 59 60 %  
72 70 77 75 34 41 46 54 52 44 41 44 54 41 40 %

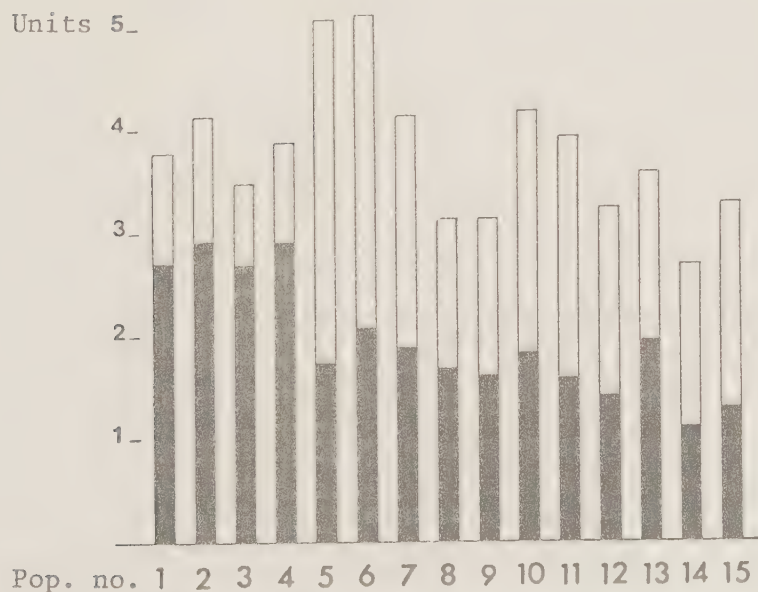


Fig. 19. Total amount of leaf hairs. Long hairs  and short hairs  in units. Above the bars, percentages of long hairs, top line, and short hairs, bottom line.

Table 26. Pollen stainability in randomly collected plants, 30 plants per population.

| Pop. no. | 0 | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100% |
|----------|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|------|
| 1        |   |   |    |    |    |    |    |    |    |    |    |    |    | 1  | 2  | 3  | 1  | 2  | 9  | 12 |      |
| 2        |   |   | 1  |    |    |    |    |    |    |    | 1  |    |    |    |    |    | 6  | 6  | 10 | 5  |      |
| 3        |   | 1 |    |    |    |    |    |    |    |    | 1  |    |    |    | 1  | 1  |    |    | 4  | 7  | 15   |
| 4        |   |   |    |    |    |    |    |    |    |    |    |    |    | 1  |    |    | 1  | 6  | 12 | 10 |      |
| 5        |   |   |    |    |    |    |    |    |    | 1  |    |    |    |    | 1  | 1  | 1  | 1  | 8  | 15 | 3    |
| 6        |   |   |    |    |    |    |    |    |    |    | 1  |    |    |    |    | 1  | 3  | 2  | 16 | 7  |      |
| 7        |   |   |    |    | 1  |    |    |    |    |    |    |    |    |    |    |    |    |    |    |    |      |
| 8        |   |   |    |    |    |    |    |    |    |    |    | 1  |    | 3  |    |    | 2  | 3  | 11 | 13 |      |
| 9        |   |   |    |    |    |    |    |    |    |    |    | 1  | 1  | 2  |    | 2  | 2  | 3  | 4  | 9  | 3    |
| 10       |   |   |    | 1  |    | 1  |    |    | 1  |    | 1  | 1  | 1  | 1  | 1  | 2  | 2  | 1  | 6  | 7  | 6    |
| 11       |   |   |    |    | 2  |    | 1  |    |    |    | 1  |    |    | 1  | 1  | 3  | 8  | 2  | 5  | 6  |      |
| 12       |   |   |    |    |    |    |    |    |    |    |    |    |    |    | 2  |    | 1  | 4  | 18 | 5  |      |
| 13       |   |   |    |    |    |    |    |    | 1  |    |    |    | 1  | 1  |    | 1  | 5  | 3  | 6  | 12 |      |
| 14       | 1 |   |    | 1  |    |    |    |    | 1  |    |    |    |    |    |    |    | 3  | 4  | 11 | 9  |      |
| 15       |   | 1 |    |    |    |    |    | 1  |    |    | 2  | 1  |    |    |    | 2  | 1  | 3  | 3  | 16 |      |

while in *ssp. scyronica* the lip was almost horizontal.

Leaf colours. Leaves of the Delphi area plants were strikingly greyish green. Analysis of variance showed high values for short leaf hairs and this undoubtedly accounts for most of the greyishness. *Melangavi* and *Megara* plants were also greyish but this was mainly due to long appressed hairs. Leaves of *ssp. argolica* and *spruneri* looked greener and sometimes especially in *spruneri*, yellowish green. Fig. 19 shows long and short hairs in arbitrary units and in percentages within each population.

Number of flowers. In the field the average number of flowers per verticillaster varied with taxa. *Spruneri* and *ssp. argolica* usually had six flowers (sometimes up to ten), *ssp. swainsonii* two to six, *ssp. melangavica* two, occasionally six, and *ssp. scyronica* always two. Greenhouse experiments showed that the environment may influence this character (in the 6-flowered taxa).

## DISCUSSION

Field observations of morphological characters, and trends in the experimental results indicated that some of the intrapopulation variation was habitat correlated. The Delphi population seemingly consists of several subpopulations, each specialized for its particular microhabitat within the population area.

Such microhabitat correlated variation has also been found in other *Stachys* species such as *ionica*, *candida*, *chrysantha*, *spreitzenhoferi*. Wilcock (1974) reported extensive variation in the leaf and inflorescence characters of *S. palustris* both within and between populations. Some patterns of variability were habitat related and at least two forms of *S. palustris* occur in nature and can be recognized by their morphological response to wet and dry environmental conditions (a marshland form and a dry-ground form). Plants grown in controlled environments showed phenotypic plasticity in leaf and rhizome characters.



Bradshaw (1965) has discussed various aspects of phenotypic plasticity.

Varied environment experiments. The varied environment experiment suggested that sun, shade and intermediate plants responded somewhat differently. Most remarkable was the 'delayed' response to the shade environments in leaf characters of the sun plants. This may have a physiological explanation. In their studies, Blackman and Rutter (1947, 1948) found that *Scilla non-scripta* was very sensitive to reduction in light intensity. This sensitivity was mainly due to the effect of light intensity on the net assimilation rate. They established that the assimilation rate was directly proportional to the logarithm of the light intensity. Fall in the assimilation rate was compensated for by an increase in leaf area. But below 0.6 of daylight the reduction in net assimilation rate was no longer balanced by a further increase in leaf area and growth was therefore reduced.

The *swainsonii* sun plants are probably adapted to a high light intensity level with a high critical compensation point. The light intensity in the shade environment may have been just below the critical point for the sun plants during the first season and may have been slightly different the following year. Another possible explanation is that the sun plants need more time to become 'adjusted' to shade and therefore did not respond with an increase in leaf size until the second season. Adaptation to different light intensity was demonstrated by Björkman and Holmgren (1963) who measured the photosynthetic activity in sun and shade ecotypes of *Solidago virgaurea*. They found that the sun ecotype could utilize intense light more efficiently than the shade type was capable of doing; the shade plants on the other hand could use weak light more efficiently. Furthermore the two ecotypes differed adaptively in their photosynthetic qualities. The results suggested that a single genotype of *S. virgaurea* would not perform adequately in both open and shady habitats.

Morphological differences between sun and shade plants have been observed in several plant species.

In his extensive investigation of *Prunella vulgaris* Böcher (1949) included varied environment experiments. Samples were cultivated, 'some in light, warmth and drought' and 'some in shade, cold and dampness'. Considerable differences resulted in size, number of flowering shoots, spike length and other characters between plants of the two environments. Differences in leaf size between light and shade plants were very conspicuous.

Böcher also examined the variation in relation to the ecological conditions of the habitat. The series from light open localities to woodlands and shady localities showed a clinal variation in all characters examined.

In *Gilia achilleifolia* Grant (1954) found a striking variation within populations. Transplant experiments proved that some characters were modified by the environment (size, pedicel length, number of flowers). Other characters were genetically determined. Progeny tests showed for instance that some individuals in the natural populations were heterozygous for genes controlling type of calyx hairs. Grant also noticed an extraordinary variation between plants in sunny and shady habitats even where those habitats were close together. Progeny tests indicated that the variability was partly hereditary and partly modificative.

Dale and Elkington (1974) found that much of the morphological variation in *Cardamine pratensis* was due to phenotypic plasticity. The habitat data indicated that most of the variation was determined by environmental factors and this was confirmed by plasticity experiments. Clones were divided and subjected to varying environmental conditions. Significant differences between treatments were obtained. Characters of the rosette leaves were extremely plastic, and soil moisture, air temperature and humidity influenced this plasticity. Variation in the number of leaflets and in the thickness of the terminal leaflet was found to be genotypic.

Morphological variation is common in plants and much has been written on this topic. But most investigations which treat habitat correlated variation concern

large areas (between populations and between regions). This is not the type of variation occurring in the *swainsonii* complex; only the variation within populations is habitat-correlated (microhabitat). Differences between populations cannot be attributed to ecological factors since all taxa grow in the same general type of habitat. It is not a matter of ecotypic subspecies as in *Gilia capitata* (Grant, 1950, 1952) or *Potentilla glandulosa* (Clausen and Hiesey, 1958).

#### Intrapopulation variation

Studies of intrapopulation variation have been sparse especially those dealing with micro-environment correlation in outbreeding perennials (as in the *swainsonii* complex). But lately an increasing interest in the subject has become evident.

Bradshaw (1959) found differentiation in *Agrostis tenuis* over a very short distance. Even though the distribution was continuous the differentiation pattern changed with the local variation in the environment. All characters observed varied continuously. From his investigation he concluded that the environment was the dominating factor in determining population differentiation.

In a number of morphological characters the populations of *Anthoxanthum odoratum* (Snaydon and Davies, 1972) from the mosaic of contrasting environments of the Park Grass Experiment, Rothamsted, showed differences which were closely correlated with changes in the environment. These differences had evolved over distances of approximately 30 m and within 50 years; they were considered to be adaptive and probably the result of natural selection acting upon genetic variation within populations. Hamrich (1979) emphasized that this study (Snaydon and Davies 1972) and that of Snaydon (1970) as well as the *Poa annua* work of Law et al. (1977) demonstrate that perennials maintain locally adapted genotypes which are correlated with subtle differences in the environment.

Silander and Antonovics (1979) examined the ecological amplitude of *Spartina*

patens along a 200 m transect through dune, swale and marsh communities. There was a clear microdifferentiation into subpopulations (dune, swale and marsh plants) in a large number of characters. Biometrical analysis of reciprocal transplantations and studies on allozymic variation (Silander, 1979) revealed genetic differences between the subpopulations.

Linhart's investigation (1974) on *Veronica peregrina* showed that a great intrapopulation variation can be found even in annual self-pollinating species. *V. peregrina* grew in and around seasonal pools. Plants from the centre and from the periphery were differentiated into subpopulations by distinctive arrays of morphological features. The variability was greater in 'center plants' than in 'periphery plants'. These differences could be understood as adaptations to different microhabitats and were perhaps maintained by disruptive selection. Electrophoretic pattern of five enzyme systems (Keeler 1978) of the same plants also revealed more variability in center plants than in periphery plants.

Allard, Jain and Workman (1968) pointed out that there is a remarkable genetic diversity within inbreeding species and that any given population contains individuals of many different genotypes. The pattern of variation reflects adaptation to the local environment. Such local differentiation appears to provide for massive storage of genetic variability.

When compared with *S. swainsonii* most of the species mentioned above differ basically in some way (breeding system, longevity, ploidy level, etc.). But these investigations all clearly point towards genetic microdifferentiation. Not only in morphological characters is this becoming evident but also in isozyme patterns as, for instance, in *Avena barbata* and *A. fatua* (Marshall and Allard 1970). Furthermore, correlations between quantitative characters and enzyme genotypes have been reported (Hamrick and Allard 1972, 1975). Likewise, studies of physiological characters as in *Agrostis tenuis* (Bradshaw 1960) and *Anthoxanthum odoratum* (Davies and Snaydon 1974) and many others, show that microdifferentiation is accorded increasing importance in the



present-day view of population variation.

The evolutionary significance of micro-environmental differentiation has been discussed by Jain and Bradshaw (1966) and Bradshaw (1972).

#### Inter-population variation

The results of the morphological character analyses among the 15 populations revealed occasional discontinuities but most characters varied continuously. Such continuous variation suggests polygenic control. Populations were grouped into five tentative taxa on the basis of combinations of characters. Single distinguishing features were extremely rare.

One of the groups, *ssp. argolica*, is extremely variable. In the Navplion area one could sometimes find plants which resembled *spruneri* (pops. 14 and 15), especially in the broadly attached floral leaves, the narrow cauline leaves and the sturdy, yellowish green appearance. In the greenhouse, however, the plants were less sturdy and the yellowish colour disappeared; these characters may therefore be environmentally influenced. Navplion also proved to be one of the most variable of the Argolis populations. At Korinthos plants were likewise extremely variable and features (e.g. number of teeth, pubescence, leaf shape) of *spruneri* and *ssp. melangavica* plants were sometimes evident.

In the north-eastern part of the Argolis Peninsula plants were sometimes difficult to distinguish and were confusingly like *ssp. swainsonii* especially in leaf shape and pubescence.

The variability within and among the Argolis populations may be evidence of hybridization. This must have occurred long ago, since the populations are now allopatric.

Plants from all areas were grown in the greenhouse and used for crossing experiments. The differences between populations as well as among progenies within populations were retained in cultivation (allowing for phenotypic plasticity). This suggests genetic variability at both inter- and intrapopulation levels.

## CONCLUSIONS

### Intrapopulation variation within *ssp. swainsonii*

The morphological variation within the *swainsonii* populations at Delphi and Arakhova has caused some taxonomic problems in the past (see Historical Outline). But the pattern of variation together with results of crossing experiments show that it is not possible to recognize any subspecific units within these populations.

Thus the following conditions in the *swainsonii* population seem to provide a system of specialization in combination with flexibility:

- (1) Apparent genetic variation within the population.
- (2) Selection pressure moulding the population in different directions according to microhabitat.
- (3) Capacity to respond phenotypically to varied micro-environments (plasticity).
- (4) A population consequently made up of several different subpopulations each specialized for a particular microhabitat.
- (5) Outcrossing promotes gene exchange thereby maintaining genetic polymorphism.
- (6) At the same time geitonogamy and vicinism act to stabilize the local gene pool.
- (7) Adequate nutlet production over several years (perennial habit).
- (8) Local nutlet dispersal close to the mother plant in a micro-environment where the chances for the plants to grow, mature and reproduce are good.
- (9) No external or internal barriers to gene exchange between subpopulations. Crosses give rise to completely fertile progeny.

### Interpopulation variation

The following conclusions were drawn from the morphological interpopulation studies of the 15 populations:

- (1) The area of distribution can be divided into five parts.
- (2) Four of these can be rather easily distinguished but the fifth part, the Argolis Peninsula, is an aggregate of differentiated populations. These are not distinct enough, however, to deserve taxonomic recognition on morphological grounds.
- (3) All populations are allopatric and therefore no gene exchange occurs between populations.

## CYTOLOGY

The aim of the cytological investigation was to determine the somatic chromosome number for all taxa in this study, and to find out if the wide range of morphological variation could be attributed to polyploidy, or if it could be correlated with meiotic irregularities and reduced pollen fertility.

### MATERIAL AND METHODS

Chromosome number was determined in root tips of greenhouse-grown plants which were raised from seeds collected in nature. Several pretreatments were tried, aesculin, colchicine and 8-hydroxyquinoline, but refrigeration at 4°C for 12 hours gave the best result. Root tips of 12-week-old seedlings were fixed in the Svalöv modification of Navashin's fixative (Müntzing 1933), embedded in paraffin, sectioned at 10  $\mu$ m, and stained in a 1% aqueous solution of crystal violet. Five to ten metaphase plates per plant were counted in at least 10 plants (10--30) per population, except for *euboica* where only 1 plant was available. Populations for which the chromosome number was determined are marked \* in the locality list in Appendix 1.

Chromosomes were also counted in artificially produced hybrids.

Meiosis in pollen mother cells was studied in squash preparations of young anthers. Buds were fixed in Carnoy II (absolute alcohol, chloroform and glacial acetic acid, 6:3:1), stored in 70% alcohol at -18°C and stained in carmine (carmine dissolved in 45% acetic acid, 4% solution). The anthers were squashed and permanently mounted in Hoyer's medium (Beeks 1955, squash technique slightly modified). Cultivated plants of all taxa as well as hybrids of many combinations



were examined. In addition buds were fixed in the field at Korinthos, where the range of variation was unusually wide and at Monemvasia, where natural hybridization between *spreitzenhoferi* ssp. *virella* and *chrysantha* was suspected.

The methyl green-pyronin method (Busch and Smetana 1970) was used to verify nucleoli in some hybrids.

#### CHROMOSOME NUMBER AND MORPHOLOGY

All subspecies of *S. swainsonii* Benth.: ssp. *swainsonii*, ssp. *scyronica*, (Boiss.) Phitos et Damboldt, ssp. *melangavica* D. Perss. and ssp. *argolica* (Boiss.) Phitos et Damboldt, have the same chromosome number,  $2n=34$ . This number was also found in all other species used in the crossing programme; they are *S. spruneri* Boiss., *S. ionica* Hal., *S. candida* Bory et Chaub., *S. chrysantha* Boiss., *S. spreitzenhoferi* ssp. *spreitzenhoferi* Heldr., *S. spreitzenhoferi* ssp. *virella* (D. Perss.), *S. euboica* Rech. f., *S. messeniaca* Boiss., *S. mollissima* Willd., *S. tetragona* Boiss. et Heldr. (Fig. 20).

The chromosomes of all taxa are small, about  $1-2\ \mu\text{m}$ , allowing for possible modification due to fixing treatment. They are rod-shaped and mostly without visible constrictions. Only rarely could satellites of one or two chromosomes be seen (in ssp. *swainsonii*, ssp. *scyronica*, *spruneri*, *messeniaca*, *mollissima* and *tetragona*). Thus, structurally, all chromosomes appear very similar and are therefore difficult to identify. This makes it almost impossible to observe any differences in the karyotypes. For the same reasons morphological details could not be studied.

Previous reports. Strid (1965) published the chromosome number  $2n=34$  for *S. swainsonii* Benth. (coll.) from Akrokorint, Sophikon and Loutraki. Phitos and Damboldt (1969) reported  $n=17$  for *S. swainsonii* ssp. *swainsonii* from the locus classicus, Delphi and Damboldt (1976) published  $2n=34$  for *S. ionica*



Fig. 20. Root-tip mitosis. A: ssp. *swainsonii*. -- B: ssp. *argolica*. -- C: ssp. *melangavica*. -- D: ssp. *scyronica*. -- E: *spruneri*. -- F: *ionica*. -- G: *euboica*. -- H: *mollissima*. -- I: *tetragona*. -- J: *candida*. -- K: *chrysantha*. -- L: ssp. *spreitzenhoferi*. -- M: ssp. *virella*. -- N: *messeniaca*. The scale is 10  $\mu$ m.

$2n=34$  was also determined for *S. euboica* Rech. f. by Runemark (in Rechinger 1961) and confirmed by Strid (1965).

The chromosome number  $2n=34$  has also been reported for other species of the genus *Stachys*, e.g. for *S. setifera*, *S. recta*, *S. maritima*, *S. lavandulaefolia*, *S. annua* and *S. menthaefolia* (Lang 1940), for *S. parviflora* (and *S. recta*) (Moore 1971) and for *S. leucoglossa* and *S. plumosa* (Koeva 1977). Morton (1973) suggested that the high basic numbers in *Stachys* (15 and 17) and some other genera of Labiatae (*Marrubium* 17, *Melittis* 15, *Nepeta* 17, *Origanum* 15, *Teucrium* 17) probably are of secondary origin due to allopolyploidy.

## MEIOSIS

Meiosis was studied in 6--24 plants/taxon in the *swainsonii* group and only 1 *euboica* plant. Meiosis was usually normal in ssp. *swainsonii*, ssp. *argolica*, ssp. *scyronica*, *spruneri*, *ionica* and *euboica* (greenhouse-grown plants); 17 bivalents were formed and univalents and laggards were rare. In a few plants of ssp. *argolica*, however, 7--15% of the tetrads showed supernumerary nucleoli: 1 or occasionally 2 additional nucleoli appeared in 1 or 2 of the tetrad's 4 nuclei and sometimes nucleoli occurred in the cytoplasm as well.

Buds of the morphologically highly variable population at Korinthos were fixed in the field. Meiosis was regular except in 2 plants where occasionally laggards occurred (in c. 2% of the cells at metaphase II and anaphase II) as well as extra nucleoli in the tetrads.

Only 6 plants of ssp. *melangavica* were analysed, 5 of which were normal. In the sixth individual meiosis was disturbed; chromosome pairing at metaphase I was reduced and 2--4 univalents were common. Laggards (2--4) occurred in c. 10% of the cells at metaphase I and II and anaphase II, and a bridge with fragment at anaphase II was recorded in 3%. Supernumerary nucleoli in the nucleus

as well as nucleoli in the cytoplasm were common at the tetrad stage. The pollen fertility of this plant was 59%.

A limited number of plants of *candida*, *chrysantha*, *ssp. spreitzenhoferi* and *ssp. virella* were studied and found to be meiotically regular. At Monemvasia buds were fixed in the field from plants suspected to be hybrids between *ssp. virella* and *chrysantha*. Univalents, laggards, and additional nucleoli at diakinesis and prophase II occurred in c. 2% and extra nucleoli appeared in 5-15% of the tetrad cells. One tetrad cell had 6 nuclei.

Some intra- and interpopulation crosses were examined. Meiosis in intra-population crosses was essentially regular and progeny of different morphological variants (3 combinations) of the Delphi population were also normal. Interpopulation crosses usually showed good chromosomal pairing with 17 bivalents at metaphase I; lagging chromosomes and extra nucleoli in the tetrads were rare (c.2%).

Meiosis in artificially produced hybrids, see Crossing experiments.

#### CHROMOSOME NUMBER IN THE HYBRIDS

The somatic chromosomes were also counted in some  $F_1$ ,  $F_2$  and  $F_3$  of all available combinations, in 16 trispecific hybrids and 4 backcrosses. All plants investigated had the chromosome number  $2n=34$ .

Only one kind of deviation, endopolyploidy, was noted -- occasional polyploid cells with 68 chromosomes were found in 3 hybrids. They all involved *ssp. spreitzenhoferi* ( $\times$  *spruneri*  $F_1$ ,  $\times$  *ionica*  $F_1$ , and *messeniacae*  $\times$  *ssp. spreitzenhoferi*  $F_2$ ).



## CROSSING EXPERIMENTS

Crossing experiments can provide valuable information about the relationship and the genetic differentiation between taxa and also contribute to the understanding of the process of speciation. An extensive crossing programme was therefore carried out.

### CROSSING PROGRAMME

The main crossing programme included 142 combinations between taxa. The direction of crossing is indicated -- the female parent is always mentioned first in a cross and *ssp. swainsonii* x *candida* and *candida* x *ssp. swainsonii*, for example, are here counted as two combinations. Some combinations represent crosses between several populations of each taxon -- in all 385 'between-population combinations' were made. The number of flowers crossed varied considerably between combinations; in some there were only a few. Altogether about 3,800 flowers were crossed.

As many populations as possible of the *swainsonii* group were intercrossed; *euboica* from Euboea and *mollissima* from Corfu were also crossed with the group.

The *swainsonii* group has a rather limited distribution in central Greece. Different species occupy ecologically similar habitats in other areas, for instance *candida*, *chrysantha*, *ssp. spreitzenhoferi* and *ssp. virella* in southern Greece (Taiyetos Oros, Parnon Oros, Kithira, Neapolis and Monemvasia). These four taxa, here referred to as the *candida* group, were crossed inter se and with the *swainsonii* group and some of the other species. Finally *messeniacae* was included in the crossing programme in spite of its distinct morphology. There were two reasons for this. The population inhabiting the

Monemvasia Peninsula was problematic and therefore the hypothesis (Greuter and Rechinger 1967) was tested that this taxon may have arisen by hybridization between ssp. spreitzenhoferi and some glandular, less hairy species. Thus messeniaca was chosen although it now has a separate distribution. Secondly, a population had been discovered south of Kalamai with suspected hybrids between candida and messeniaca. Since candida grows in the vicinity hybridization seemed likely and was therefore tried experimentally. Furthermore messeniaca was crossed with some of the other species.

In addition to the main crossing programme tetragona was crossed with some of the taxa. Since tetragona presents no taxonomic problems it was dealt with only briefly.

Finally some crosses involving three taxa were made as a means to investigate the possible origin of the populations growing between Loutraki and Akr. Melangavi which seemed to comprise a separate entity.

Populations used in the crossing experiments and results of the crosses are listed in the Appendix.

## MATERIAL AND METHODS

Plants used in the crossing experiments were raised in the greenhouse from seed collected in the field. The flowers are protandrous but, as a safety precaution, all flowers used as females in experiments were emasculated at the bud stage with a small pair of scissors and the buds enclosed in bags. When the stigma had matured after about a week, the bag was removed, pollen from the selected male parent was transferred to the receptive surface and the flowers were isolated. Pollination was repeated either one or two days later. After 10-14 days the bags were removed so that the nutlets could develop under more natural conditions. When they began to darken, the flowers were again isolated to prevent loss of seed. In successful crosses the nutlets ripened 8-10 weeks after pollination. Most taxa have 6-flowered verticillasters. Two opposite flowers open

first, then the remaining four more or less simultaneously. The first two flowers were cut off and the remaining four buds emasculated and used for crossings. The rest of the inflorescence was discarded. Emasculatation and pollination (also self-pollination of the hybrids) were performed under a stereo microscope.

The procedure was time consuming (especially since each flower produces a maximum of only 4 nutlets) but fortunately the plants flowered abundantly over a long period of time. Also they are perennials and crosses and pollinations of hybrids could therefore be repeated during the following seasons. The seeds were stored in a cool place for a few months, then sown in sterilized soil in the greenhouse. After 3--4 weeks tiny seedlings appeared and 2--3 weeks later they were transferred to individual pots. The pots were placed at random so that the effects of environmental variation would be minimized.

#### Artificial self-pollination

Hybrid flowers were isolated at the bud stage and artificially selfed, then treated as the crosses.

#### Pollen fertility

Pollen fertility was estimated by the percentage of well-developed pollen grains stained with lactophenol-aniline blue (pollen stainability). From each plant 200 pollen grains were counted usually on two different occasions.

#### Seed set

Seed set was expressed as percentage of maximum production (4 nutlets per flower) of well-formed nutlets. Many combinations produced well-formed nutlets, others small shrivelled nutlets and some no nutlets at all. After artificial pollination of pure taxa the mean seed set was less than 4 nutlets and this should be kept in mind when the hybrid yields are interpreted. The values for

pure taxa in Table 31 represent seed set after emasculation and cross-pollination -- the same treatment as in crosses -- and in Table 38 seed set after self-pollination -- since the hybrids were self-pollinated. In cultivation the seed set was below maximum maybe partly as a consequence of isolation in bags. All plants were treated in the same manner by the same person (the author) to minimize the effects of technical inconsistencies. The general conditions of the plant may also be important and some hybrids seemed to tolerate isolation in bags better than others. The seed production is an extremely complex process and the causes of reduction in output or complete failure are many. The results should therefore be looked upon as only a rough estimate of barriers to gene exchange. Although morphologically not identical the terms 'nutlet' and 'seed' are used interchangeably for instance in 'seed set'.

#### FERTILITY IN PARENTAL TAXA

Preliminary tests were performed on the parent taxa to give a basis for comparing the fertility of hybrids.

Emasculated non-pollinated flowers were isolated. None of them yielded any nutlets and it was thus assumed that autogamous agamospermy does not occur in these taxa.

Each taxon was tested for self-compatibility. Emasculated flowers were pollinated by flowers on the same plant. The average amount of seed per taxon ranged from 45 to 87.5%. After cross-pollination of emasculated flowers the output was 45 to 82.5%. Seed set after self- and cross-pollination varied slightly from taxon to taxon but was not consistently higher or lower after one kind of pollination (Table 30).

All taxa were also tested for automatic self-fertilization. Plants were isolated but evidently no selfing took place since no seeds were produced. Some slight swelling of ovaries was observed in two flowers on one *ionica* plant, but no nutlets developed. Although *ssp. scyronica* and *ssp. melangavica* have a com-



paratively short and less exerted pistil, did self-pollination not occur.

Germination. Germination of nutlets collected in the field was very erratic within each taxon. The percentages for the various taxa ranged from 24-60% (Table 27). The seeds were sown in soil and since the germination was only moderate, agar was tried. The results were no better and a number of seedlings died when transferred to soil. Soil was therefore used for germinations of all hybrid seeds. Nutlets sown in the autumn gave about the same germination percentages as nutlets sown in early February after a few months storage in a cool place. No chemical or mechanical methods were tried to improve germination.

Germination of seeds of intra- and interpopulation crosses was also variable and moderate.

Pollen stainability. Plants used as parents in the crossing experiments had a pollen stainability of > 90%, rarely 87-90%.

In intra- and interpopulation crosses, pollen stainability was usually good -- mostly > 90%, sometimes 80-90%, occasionally 60-79%; only one plant was completely sterile -- a ssp. *melangavica* combination.

Pollen stainability in interpopulation crosses of ssp. *argolica* is shown in Table 28. The last cross, *Dhrepanon* x *Methana*, which differed from the others by its low stainability, was investigated in further generations (Table 29). The  $F_2$  spanned the entire percentage range -- a phenomenon common in interspecific hybrids. About 40-50% of the pollinated hybrids gave a rather good seed set but the rest gave a poor yield comparable to that of interspecific hybrids. The parents differed morphologically also, especially in leaf shape and pubescence. Interpopulation crosses of ssp. *swainsonii* gave (73,83)87-99%. Crosses between variants within the Delphi-population had normal fertility.

Seed set. Seed set in the parents are presented in Table 30. See also Material and methods under seed set.

Table 27. Germination of hybrid seeds (main crossing programme). Germination % of seeds from natural populations included for comparison.

|              | Mean germ.% seeds from nat. pop. | Seeds, set after cross-poll. |              |                   |                                 | F <sub>1</sub> seeds |              |                   | F <sub>2</sub> seeds |              |                   | No. of comb. where germ. failed |
|--------------|----------------------------------|------------------------------|--------------|-------------------|---------------------------------|----------------------|--------------|-------------------|----------------------|--------------|-------------------|---------------------------------|
|              |                                  | Germ.% mean                  | Germ.% range | No. of comb. sown | No. of comb. where germ. failed | Germ.% mean          | Germ.% range | No. of comb. sown | Germ.% mean          | Germ.% range | No. of comb. sown |                                 |
| ssp. swain.  | 38                               | 30                           | 17-66        | 11                |                                 | 43                   | 0-100        | 9                 | 23                   | 0-67         | 6                 | 2                               |
| ssp. scyr.   | 27                               | 54                           | 33-66        | 10                |                                 | 42                   | 0-54         | 7                 | 29                   | 0-33         | 4                 | 1                               |
| ssp. mel.    | 65                               | 38                           | 0-93         | 9                 | 3                               | 50                   | 0-69         | 5                 | 29                   | 0-100        | 4                 | 1                               |
| ssp. argol.  | 45                               | 55                           | 15-90        | 12                |                                 | 44                   | 0-100        | 11                | 32                   | 0-100        | 9                 | 2                               |
| spruneri     | 56                               | 32                           | 20-77        | 5                 |                                 | 39                   | 18-60        | 4                 | 31                   | 21-36        | 4                 |                                 |
| ionica       | 38                               | 48                           | 22-80        | 11                |                                 | 37                   | 0-64         | 10                | 26                   | 17-42        | 5                 |                                 |
| euboica      | -                                | 65                           | 43-80        | 9                 |                                 | 50                   | 0-100        | 9                 | 63                   | 33-100       | 3                 |                                 |
| candida      | 47                               | 50                           | 22-95        | 12                |                                 | 28                   | 0-100        | 12                | 17                   | 0-100        | 5                 | 1                               |
| chrysantha   | 55                               | 41                           | 7-100        | 10                |                                 | 33                   | 0-64         | 8                 | 6                    | 4-13         | 1                 |                                 |
| ssp. spreit. | 24                               | 57                           | 15-79        | 12                |                                 | 26                   | 0-55         | 10                | 31                   | 0-44         | 8                 | 2                               |
| ssp. virella | 60                               | 59                           | 0-84         | 11                | 1                               | 33                   | 0-100        | 9                 | 28                   | 23-100       | 5                 |                                 |
| messeniaca   | 58                               | 57                           | 20-86        | 11                |                                 | 37                   | 0-78         | 9                 | 28                   | 17-33        | 4                 |                                 |
| mollissima   | 54                               | 59                           | 0-100        | 11                | 5                               | 26                   | 0-41         | 3                 | 0                    |              | 1                 | 1                               |

Table 28. Pollen stainability in interpopulation crosses ( $F_1$ ) of *ssp. argolica*. The designations AB, AF, AD and AE indicate different populations in the Dhrepanon area.

|                          | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 % |
|--------------------------|----|----|----|----|----|----|----|----|----|----|-------|
| Dhrepanon AB x Dhrep. AF |    |    |    |    |    |    |    |    |    | 5  |       |
| Navplion x Idhra         |    |    |    |    |    |    | 3  | 1  |    | 1  |       |
| Idhra x Dhrepanon AD     |    |    | 1  |    |    |    | 1  | 1  | 3  | 4  |       |
| Idhra x Navplion         |    |    |    |    |    |    | 1  | 1  | 2  |    |       |
| Methana x Idhra          |    |    |    |    |    |    |    |    | 1  | 5  |       |
| N. Epidhavros x Idhra    |    |    |    |    |    | 2  | 2  |    | 2  | 6  |       |
| Vromolimni x Irion       |    |    |    |    |    |    |    |    |    | 5  |       |
| Dhrepanon AE x Methana   | 2  | 1  | 1  | 1  |    |    |    | 1  |    |    |       |

Table 29. Percentage pollen stainability and seed set (*italics*) in Dhrepanon AE x Methana, *ssp. argolica* interpopulation cross.

|       |    |                 |                  |                |                   |                |                   |                                                                         |
|-------|----|-----------------|------------------|----------------|-------------------|----------------|-------------------|-------------------------------------------------------------------------|
| $F_1$ | 50 | 52<br><i>0</i>  | 57<br><i>3.8</i> | 61             | 68<br><i>15.2</i> |                | 87<br><i>12.5</i> |                                                                         |
| $F_2$ | 0  | 48<br><i>23</i> | 48               | 52<br><i>0</i> | 57<br><i>0</i>    | 91<br><i>0</i> | 96<br><i>0</i>    | 5 58 62<br><i>10</i> 84<br><i>1</i> 94 96 98<br><i>15</i> 0 <i>15.8</i> |
| $F_3$ |    |                 |                  |                |                   |                | 89                | 70 91<br><i>5.4</i> 94<br><i>43.3</i>                                   |
| $F_4$ |    |                 |                  |                |                   |                |                   | 90                                                                      |

Table 30. Seed set (%) in parental taxa.

|                      | After<br>self-<br>poll. | Within pop. | Between pop. | No. of<br>pop. in<br>cult. |
|----------------------|-------------------------|-------------|--------------|----------------------------|
| ssp. swainsonii      | 50                      | 67.5        | 75           | 12                         |
| ssp. scyronica       | 50                      | 52.5        | —            | 1                          |
| ssp. melangavica     | 87.5                    | 45          | 70           | 5                          |
| ssp. argolica        | 45                      | 55.9        | 75           | 17                         |
| spruneri             | 87.5                    | 75          | 85           | 4                          |
| ionica               | 77.5                    | 82.5        | 70           | 9                          |
| euboica              | 70                      | —           | —            | (1)                        |
| candida              | 57.5                    | 72.5        | 75           | 11                         |
| chrysantha           | 50                      | 57.5        | 75           | 18                         |
| ssp. spreitzenhoferi | 70                      | 75          | 87.5         | 6                          |
| ssp. virella         | 50                      | 70          | —            | 1                          |
| messeniaca           | 52.5                    | 50          | 60           | 7                          |
| mollissima           | 62.5                    | 57.5        | 50           | 9                          |

## FERTILITY TESTS IN THE HYBRIDS

The degree of cross-compatibility between taxa was estimated by: (1) seed set after cross-pollination; (2) germination of hybrid seeds; (3) viability and morphology of  $F_1$  hybrids; (4) pollen stainability in  $F_1$ ; (5) seed set in  $F_1$ ; (6) germination, morphology, pollen stainability and seed set in  $F_2$ ; (7) observations on later generations; (8) meiosis.

## Seed set after cross-pollination

The main crossing programme included 142 combinations and nutlets were obtained in most of them. Only 8 combinations failed (ssp. swainsonii x mollissima; ssp. scyronica x mollissima; spruneri x ionica, euboica, chrysantha, virella; euboica



x mollissima; chrysantha x ionica) but few flowers were pollinated in these combinations.

The seed set varied considerably in the different combinations. Table 31 is condensed from data in the Appendix and the number of nutlets per flower expressed in percentage of maximum nutlet production (4 nutlets = 100%). The seed set for 'pure' taxa was obtained after emasculation and cross-pollination within populations.

Table 31. Seed set after cross-pollination. Number of nutlets per flower is expressed as percentage of maximum production; 4 nutlets = 100%. - no flowers pollinated. Pure taxa were also emasculated and cross-pollinated. No value available for euboica since the plants in cultivation originate from the same individual (emasc. and self-poll. 70%). In all crosses where seed set was 0, only 5 flowers or less were pollinated.

| <div> <div>female</div> <div>male</div> </div> | ssp. swainsonii | ssp. scyronica | ssp. melangavica | ssp. argolica | spruneri | ionica | euboica | candida | chrysantha | ssp. spreit. | ssp. virella | messeniac | mollissima |
|------------------------------------------------|-----------------|----------------|------------------|---------------|----------|--------|---------|---------|------------|--------------|--------------|-----------|------------|
| ssp. swainsonii                                | 67.5            | 27.6           | 42.1             | 56.2          | 35.4     | 25.0   | 46.1    | 49.3    | 42.9       | 32.6         | 42.9         | 43.3      | 0          |
| ssp. scyronica                                 | 40.9            | 52.5           | 50.0             | 27.5          | 17.6     | 54.2   | 22.2    | 40.0    | 21.7       | 28.6         | 37.5         | -         | 0          |
| ssp. melangavica                               | 40.0            | 44.0           | 45.0             | 71.4          | 87.5     | 21.2   | 12.5    | 37.5    | 37.5       | -            | -            | -         | 25.0       |
| ssp. argolica                                  | 47.8            | 41.0           | 58.3             | 55.9          | 52.4     | 32.8   | 35.5    | 60.9    | 23.1       | 16.2         | 83.3         | 33.9      | 35.0       |
| spruneri                                       | 45.2            | 20.6           | 25.0             | 58.3          | 75.0     | 0      | 0       | 27.8    | 0          | -            | 0            | -         | -          |
| ionica                                         | 60.0            | 33.0           | -                | 26.0          | 12.8     | 82.5   | 19.1    | 76.9    | 9.1        | 19.8         | 10.0         | 4.8       | 16.1       |
| euboica                                        | 33.0            | 31.7           | 25.0             | 33.3          | 60.0     | 38.5   |         | 17.9    | 37.5       | -            | 36.4         | -         | 0          |
| candida                                        | 55.8            | 18.5           | 31.2             | 46.3          | 18.3     | 18.8   | 59.4    | 72.5    | 36.4       | 30.8         | 12.5         | 61.1      | 52.8       |
| chrysantha                                     | 24.1            | 44.0           | -                | 43.4          | 37.5     | 0      | 30.0    | 23.9    | 57.5       | 6.9          | 13.9         | 45.8      | 7.1        |
| ssp. spreit.                                   | 34.1            | 61.4           | 27.3             | 30.6          | 47.9     | 33.3   | 22.4    | 23.0    | 23.3       | 75.0         | 36.7         | 32.4      | 13.6       |
| ssp. virella                                   | 19.3            | 16.7           | -                | 21.0          | 12.5     | 23.6   | 67.3    | 16.7    | 21.6       | 31.3         | 70.0         | 10.4      | 13.5       |
| messeniac                                      | 59.6            | 14.4           | 16.7             | 58.8          | 37.0     | 21.2   | 38.9    | 26.6    | -          | 47.0         | 45.0         | 50.0      | 34.6       |
| mollissima                                     | 28.1            | 58.3           | 22.2             | 39.3          | 59.0     | 8.3    | 8.3     | 17.9    | 8.3        | 3.6          | 12.5         | -         | 57.5       |

Cross-pollinations within the swainsonii-group gave relatively good seed set. Values within the candida-group were somewhat lower than those of the swainsonii-group except candida x chrysantha and ssp. spreitzenhoferi x ssp. virella. A few crosses between the two groups gave unexpectedly good yields: candida x ssp. swainsonii and reciprocal; candida x ssp. argolica; ssp. spreitzenhoferi x ssp. scyronica; and ssp. argolica x ssp. virella. Reciprocal differences in combining ability were not pronounced. Where apparent differences occur (euboica x spruneri 60%, reciprocal combination 0; ionica x candida 76.9%, recip. comb. 0; candida x mollissima 52.8%, recip. comb. 17.8%) this may partly be attributed to the few flowers pollinated.

#### Germination of hybrid seeds

Nutlets of the 134 successful crosses germinated and gave rise to hybrid seedlings in all but 9 combinations (ssp. melangavica x ionica, candida, chrysantha; ssp. virella x spruneri; mollissima x ssp. scyronica, ionica, candida, chrysantha, ssp. spreitzenhoferi).

Germination was very variable. Table 27 which also includes  $F_1$  and  $F_2$  shows germination data which are summarized from detailed data in the Appendix.

Germination of hybrid seeds does not seem to be correlated with pollen fertility in  $F_1$  plants (Fig. 21).

#### Hybrid inviability

Most  $F_1$  hybrids grew into vigorous plants which reached at least the normal size of the smallest parent. But some plants died at the seedling stage, others within 3-5 months i.e. before reaching maturity, still others were dwarfed. This pattern was repeated in  $F_2$  and  $F_3$ . Table 32 shows the mortality and the number of dwarfed plants in  $F_1$ ,  $F_2$  and  $F_3$ .

Usually only one or two plants of the same combination fell within one category. The abnormalities were distributed in hybrids of all taxa but were more frequent

% pollen stainability in  $F_1$

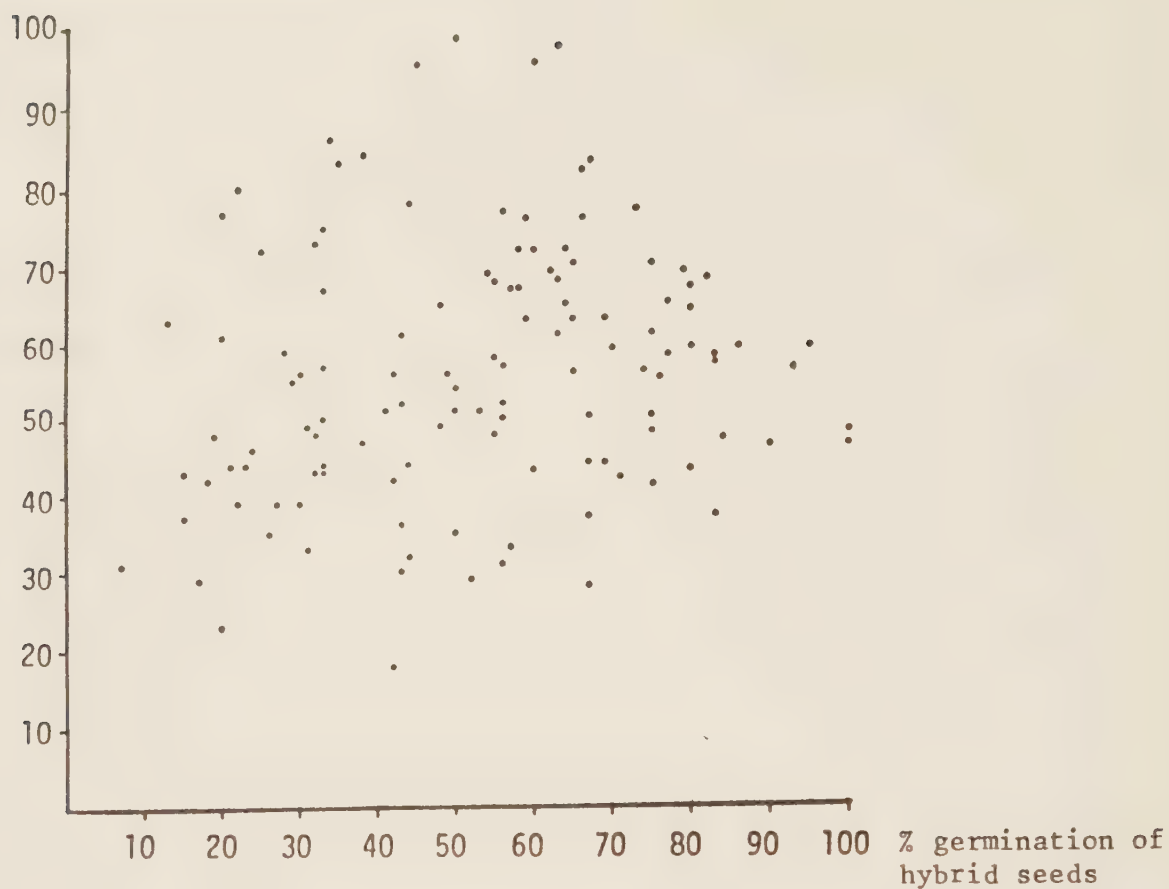


Fig. 21. Pollen stainability in  $F_1$  plotted against germination of hybrid seeds set after cross-pollination.

Table 32. Mortality and number of dwarfed plants.

|       | No. of<br>comb.<br>where<br>nut-<br>lets<br>germi-<br>nated | No. of<br>comb.<br>where<br>seedl.<br>death<br>occurred | % of<br>total<br>no. of<br>germ.<br>comb. | Total<br>no. of<br>seedl.<br>deaths | % of<br>total<br>no. of<br>seedl. | No. of<br>comb.<br>where<br>deaths<br>occurred<br>within<br>3-5 mo. | % of<br>total<br>no. of<br>germ.<br>comb. | Total<br>no. of<br>deaths | % of<br>total<br>no. of<br>seedl. | No. of<br>comb.<br>where<br>dwarfs<br>occurred | % of<br>total<br>no. of<br>germ.<br>comb. | Total<br>no. of<br>dwarfs | % of<br>total<br>no. of<br>seedl. |
|-------|-------------------------------------------------------------|---------------------------------------------------------|-------------------------------------------|-------------------------------------|-----------------------------------|---------------------------------------------------------------------|-------------------------------------------|---------------------------|-----------------------------------|------------------------------------------------|-------------------------------------------|---------------------------|-----------------------------------|
| $F_1$ | 125                                                         | 16                                                      | 12.8                                      | 29                                  | 1.9                               | 19                                                                  | 15.2                                      | 19                        | 1.2                               | 19                                             | 15.2                                      | 32                        | 2.1                               |
| $F_2$ | 85                                                          | 6                                                       | 7.0                                       | 6                                   | 1.0                               | 18                                                                  | 21.2                                      | 23                        | 3.6                               | 9                                              | 10.6                                      | 12                        | 1.9                               |
| $F_3$ | 49                                                          | 16                                                      | 32.7                                      | 18                                  | 7.4                               | 10                                                                  | 20.4                                      | 14                        | 5.8                               | 17                                             | 34.7                                      | 24                        | 9.8                               |

in those involving *ssp. argolica* and *ssp. spreitzenhoferi* -- 9 of the 19 deaths after 3-5 months in  $F_1$  were *argolica* crosses (within the *swainsonii*- and with the *candida*-groups) and 12 of the 32 dwarfed  $F_1$  plants were *spreitzenhoferi* crosses (with the *swainsonii* group). Compared with the total number of hybrids, the loss must be considered small.

#### Morphology of the $F_1$ hybrids

Most of the hybrids developed well. In general appearance they were intermediate between the parents. But individual characters were sometimes inherited from one parent only, for instance in *ssp. argolica* x *ssp. virella* where the colours of corolla and stamens and the lower lip pattern were those of *ssp. virella* (deep purplish red). *Ssp. spreitzenhoferi* has the same kind of flowers as *ssp. virella* and *ssp. argolica* x *ssp. spreitzenhoferi* inherited colour characters in the same manner.

The two subspecies of *spreitzenhoferi* differ in leaf characters and this was also evident in the hybrids; *ssp. spreitzenhoferi* hybrids had hairy, more ovate leaves while *ssp. virella* hybrids had less hairy but glandular and more rounded leaves with the characteristic smell of *ssp. virella*. In *ssp. scyronica* x *ionica* the floral leaves had petioles as in *ionica* and the upper lip had purplish-red marks as in *ssp. scyronica*. This upper lip character appeared in all *ssp. scyronica* hybrids in  $F_1$  (except in one of the *euboica* x *ssp. scyronica* crosses) but segregated in  $F_2$ .

Characteristic odours as in *euboica* and *mollissima* were usually strong in the hybrids. *Chrysantha*, the only yellow-flowered species, yielded hybrids with more or less yellow flowers, occasionally white.

Hybrids of *ssp. swainsonii* and *ssp. argolica* with a common parent were compared morphologically for the purpose of clarifying character differences between the two subspecies. Since *ssp. argolica* is extremely variable and sometimes *swainsonii*-like it was often difficult to distinguish between a pair of hybrids. Usually the leaves of *swainsonii*-hybrids were somewhat more ovate



with crenate margin as opposed to more elliptic with dentate margin (but of course the common parent also influenced these characters). The short hairs of ssp. *swainsonii* often made the leaves slightly greyish. Sometimes only one of these character differences was noticeable.

At the other extreme of the variation range of ssp. *argolica*, plants with characters resembling those of *spruneri* were found in natural populations. Traits such as triangular floral leaves and narrow cauline leaves often appeared in hybrids of such *argolica* plants.

Ssp. *swainsonii* x ssp. *argolica* (and the reverse) were often difficult to recognize on morphological characters. If the greenhouse effect was ignored, many of these hybrids resembled plants found in natural populations especially on the Argolis-peninsula.

This resemblance was also noted in hybrids of *spruneri* x ssp. *argolica* and reciprocal crosses.

Abnormalities were few in  $F_1$ : deformed corolla < 1% of the total number of  $F_1$  hybrids; one flower with two pistils in one plant of *mollissima* x ssp. *swainsonii*; one peloria in one ssp. *virella* x ssp. *argolica*; deviation in corolla colour about 1%, either a greenish band or spot on the upper lip (ssp. *scyronica* and ssp. *spreitzenhoferi* hybrids mainly) or lack of purplish pattern on the lower lip.

#### Pollen stainability in the $F_1$ hybrids

Pollen stainability was more or less reduced in the hybrid combinations with a few exceptions and the range within combinations was very wide (Fig. 22). Table 33 lists the pollen stainability of reciprocal combinations in  $F_1$  and  $F_2$ .

The *swainsonii*-group. In hybrids within *S. swainsonii*, mean values ranged from 59 to 85% and was lowest in ssp. *swainsonii* x ssp. *argolica*. There seemed to be definite fertility barriers between these two subspecies. The

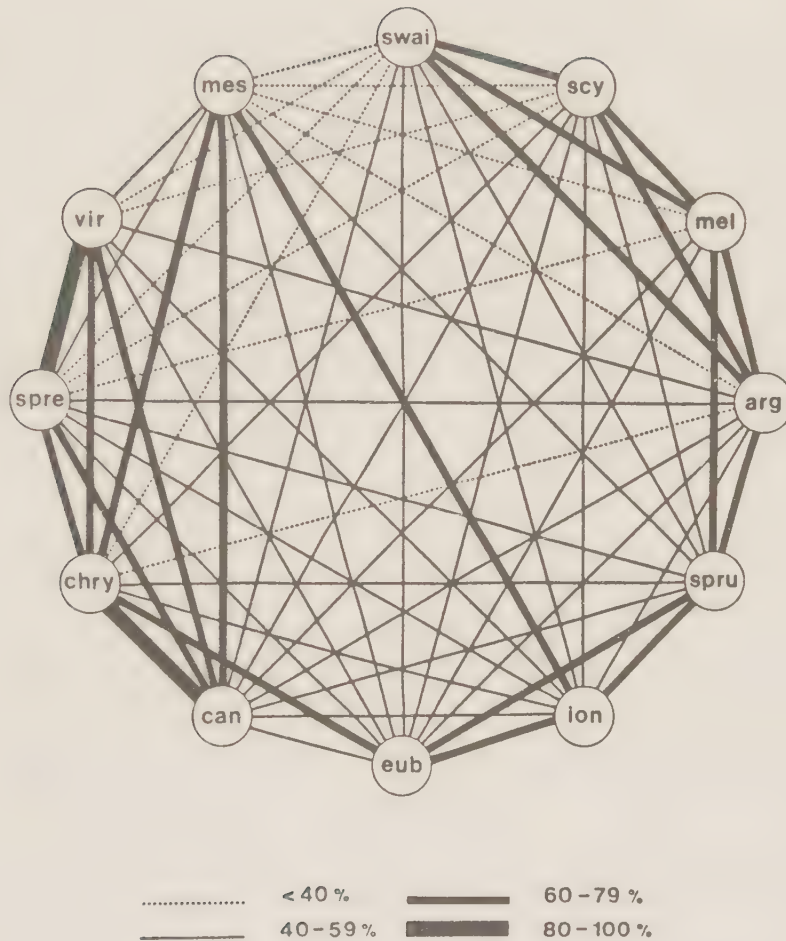


Fig. 22. Pollen stainability in  $F_1$ . Taxa are indicated by the first letters of the specific and subspecific name. Each line represents the average of the mean values for all crosses between two taxa.



|                       |         |                |                |          |          |                |          |                |             |  |
|-----------------------|---------|----------------|----------------|----------|----------|----------------|----------|----------------|-------------|--|
| ssp. argolica         |         |                |                |          |          |                |          |                |             |  |
| x candida recip.      | 9<br>35 | 84<br>90       | 60-99<br>67-99 | 43<br>56 | 5<br>23  | 28-65<br>29-72 | 65<br>44 | -<br>11-62     | 1<br>8      |  |
| x chrysanth           | 6<br>4  | 66<br>43       | 56-71<br>21-86 | 28<br>48 | 7<br>5   | 20-42<br>35-58 |          |                | 0           |  |
| x ssp. spreit. recip. | 5<br>1  | 73<br>56       | 68-77<br>-     | 50<br>56 | 4<br>32  | 37-58<br>42-73 | 57<br>48 | 20-93<br>36-87 | 13(14)<br>7 |  |
| x ssp. virella recip. | 24<br>8 | 18-67<br>46-66 |                | 46<br>57 | 24<br>8  |                | 59       | 51-69          | 3<br>0      |  |
| spruneri              |         |                |                |          |          |                |          |                |             |  |
| x candida recip.      | 2<br>13 | 53<br>61       | 40,66<br>45-91 | 61<br>39 | 2<br>3   | 59,63<br>21-51 | 53       |                | 0<br>1      |  |
| x chrysanth           | 0<br>1  | 43-71          |                | 57       | 15       |                | -        |                | 1           |  |
| x ssp. spreit. recip. | 0<br>0  | 47-85          |                | 63       | 6        |                | 77       | 54-92          | 6           |  |
| x ssp. virella recip. | 0<br>1  |                |                |          | 0        |                |          |                |             |  |
| ionica                |         |                |                |          |          |                |          |                |             |  |
| x candida recip.      | 0<br>3  | 65             | 25-92          | 58<br>43 | 10<br>1  | 45-80          | 68       | -              | 1(2)<br>0   |  |
| x chrysanth           | 0<br>0  |                |                | 56       | 5<br>0   | 33-75          | 66       | -              | 1           |  |
| x ssp. spreit. recip. | 0<br>0  |                |                | 48<br>50 | 6<br>43  | 29-66<br>26-76 | 67       | 55-85          | 0<br>10     |  |
| x ssp. virella recip. | 0<br>9  | 75             | 26-95          | 61<br>51 | 3<br>9   | 44-75<br>30-77 |          |                | 0<br>0      |  |
| euboica               |         |                |                |          |          |                |          |                |             |  |
| x candida recip.      | 2<br>2  | 83             | 80,85          | 43<br>68 | 1<br>26  | -<br>39-92     | 59<br>53 | 45,72<br>10-92 | 2<br>10     |  |
| x chrysanth           | 0<br>0  |                |                | 83<br>58 | 2<br>10  | 82,84<br>39-78 | -        |                | 1<br>0      |  |
| x ssp. spreit. recip. | 0<br>0  |                |                | 50       | 0        | 22-99          |          |                | 0           |  |
| x ssp. virella recip. | 0<br>0  |                |                | 56<br>47 | 10<br>20 | 22-93<br>21-80 | 48<br>74 | -<br>59-87     | 1<br>4      |  |



barriers varied in strength between different populations as shown in Fig. 23. Delphi x Nea Epidhavros and Delphi x Methana had the highest stainability. In general plants in the NE--E part of the Argolis peninsula were morphologically more similar to *ssp. swainsonii* than were plants in the Navplion-Dhrepanon area. In Fig. 22 the relationship between taxa of *swainsonii* are illustrated by lines, 60-79%, but each line represents the average of the means for all crosses between two taxa. The value for *ssp. swainsonii* x *ssp. argolica* fell at the lower end of the interval, the others toward the higher.

*Spruneri* x *ssp. argolica* had the highest pollen stainability among the *spruneri* hybrids. There was also reciprocal differences in this combination (comments below).

*Ionica* hybrids with *S. swainsonii* were only semi-fertile (in the 40's and 50's).

*Euboica* x *spruneri* and *euboica* x *ionica* had a higher fertility in  $F_1$  than did crosses with the *swainsonii* group.

The *candida* group. The cross *candida* x *chrysantha* yielded completely fertile progeny and so did *ssp. spreitzenhoferi* x *ssp. virella* with a few exceptions. *Ssp. spreitzenhoferi* x *messeniacae* hybrids were only semi-fertile and morphologically not closely resembling the *Monemvasia* population. (see pp. 104--105). On the other hand *candida* x *messeniacae* had higher fertility and was more like the suspected natural hybrids (see p. 105).

Between the two groups. Pollen stainability was much reduced in  $F_1$  hybrids between the groups.

Fig. 24 shows the pollen stainability in  $F_1$  *mollissima* hybrids (for details see Appendix 2).

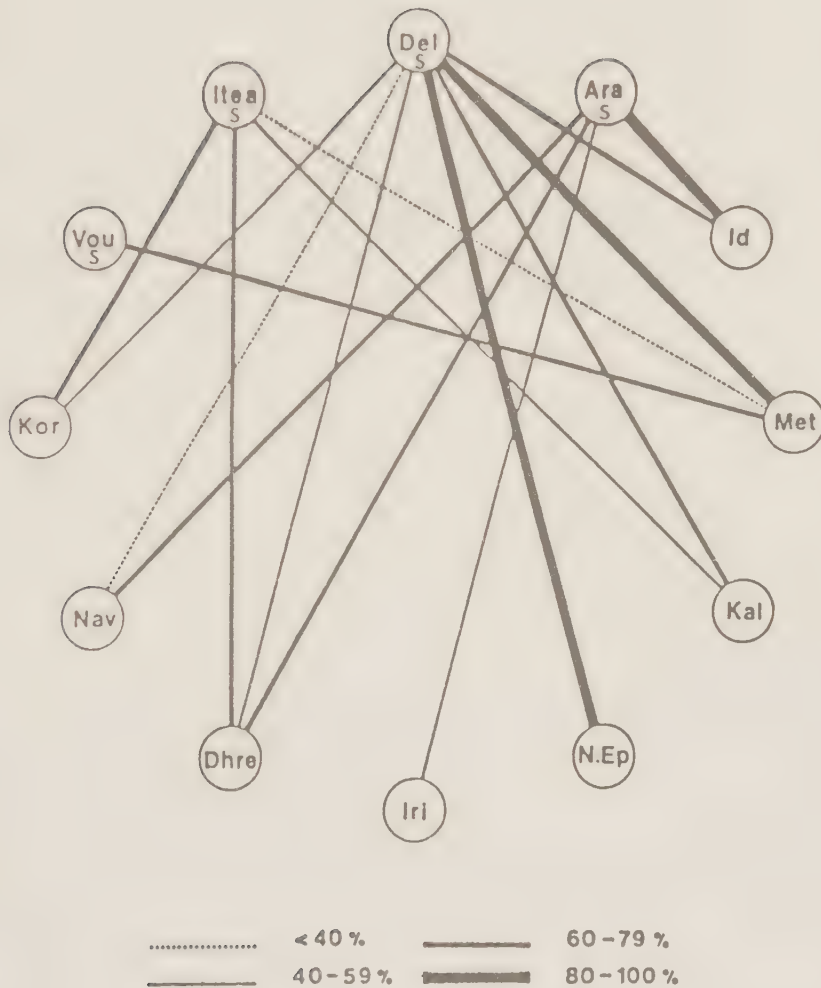


Fig. 23. Pollen stainability in *ssp. swainsonii* x *ssp. argolica* hybrids. Populations are identified by the first letters of the geographical source. Those marked s are *ssp. swainsonii* populations (Vounhiora, Itea, Delphi and Arakhova) all others are *ssp. argolica* (Idhra, Methana, Kalloni, Nea Epidhavros, Irion, Dhrepanon, Navplion and Korinthos).

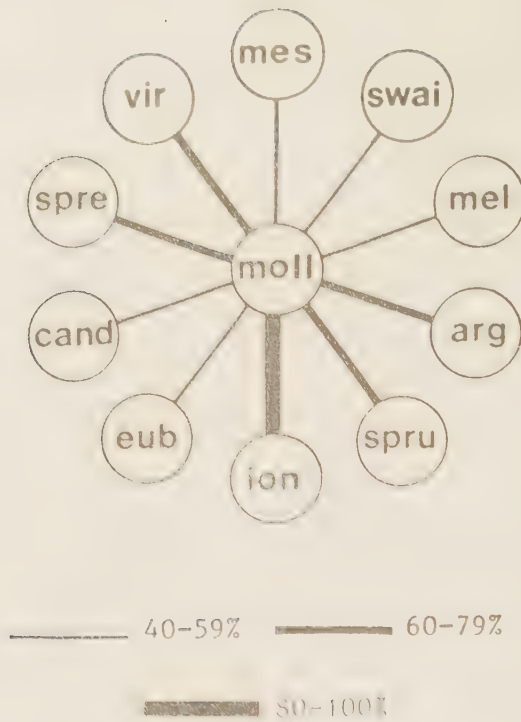


Fig. 24. Pollen stainability in  $F_1$  mollissima hybrids.

#### Reciprocal differences in pollen stainability

Differences in pollen stainability between reciprocal combinations were usually not conspicuous (Table 33) but those involving spruneri and ssp. argolica showed increasingly greater differences in later generations (mean values for spruneri x ssp. argolica:  $F_1$  86,  $F_2$  88,  $F_3$  79,  $F_4$  93%; for ssp. argolica x spruneri: 69, 56, 49, 22%) and are described here in detail.

Seed set after artificial cross-pollination was good in both combinations, 58.3 and 52.4 %. All crosses attempted produced nutlets but in four crosses they were few and did not germinate (Korinthos x Akraifnion, Kaza x Methana, Akraifnion x Navplion, Parnis x Dhrepanon). Pollen stainability and seed set in the plants that produced offspring are shown in Tables 34 (spruneri x ssp. argolica) and 35 (ssp. argolica x spruneri).

Spruneri x ssp. argolica: Four population combinations gave 22  $F_1$  plants. In three of these population combinations the pollen stainability in  $F_1$  was > 91%

Table 34. Percentage pollen stainability and seed set (*italics*) in spruneri x ssp. argolica. - no flowers.

|                | No 208-2                      | 208-8                                                  | 208-3     | 211-6            | 211-5               | 212-1                     |
|----------------|-------------------------------|--------------------------------------------------------|-----------|------------------|---------------------|---------------------------|
| F <sub>1</sub> | 92<br>17.4                    | 94<br>40.6                                             | 98<br>2.9 | 88<br>0.6        | 97<br>2.6           | 98<br>5.6                 |
| F <sub>2</sub> | - 66 93 93 97<br>5.8 3 25 2.6 | - - 95 96 97 97 98 98<br>60 18.2 0 17.3                | 98        | 90 96<br>2.1 6.2 | 46 85 94 95<br>33.3 | 63 64 93 95 98<br>0 0 0 0 |
| F <sub>3</sub> | 86 89 90<br>50.9 7.9          | - - 61 89 95 98 99 99 99 99 100<br>47.9 46.9 64.8 51.9 | 1         | 4 57 73          |                     |                           |
| F <sub>4</sub> | 88 95 88 95 96                | 97 98 99 99 83 92 98 65 98 98 98 99                    |           |                  |                     |                           |

Table 35. Percentage pollen stainability and seed set (*italics*) in ssp. argolica x spruneri. - no flowers.

|                | No 133-2                                           | 133-1                    | 133-3                                    | 133-5        | 134-2     |
|----------------|----------------------------------------------------|--------------------------|------------------------------------------|--------------|-----------|
| F <sub>1</sub> | 53<br>22.2                                         | 72<br>5                  | 74<br>17.4                               | 52<br>36     | 99<br>0.7 |
| F <sub>2</sub> | 26 35 36 50 51 54 57 65 70 88<br>16.7 11.1 1.2 8.3 | 62 69 78<br>25 7.9       | 0 24 30 43 44 50 58 87<br>0 5.8 9.4 37.5 | 32 94<br>0   | 88<br>0   |
| F <sub>3</sub> | 56 0 25 41<br>10                                   | 64 - - 0 72 92<br>0 0 50 | 41                                       | 66 88<br>0 0 |           |
| F <sub>4</sub> | 18 26                                              |                          |                                          |              |           |



in all plants except one (88 %), in  $F_2$  it was mostly high but in  $F_3$  reduced. The fourth population combination (no. 208) produced 8  $F_1$  plants with pollen stainability 1, 2, 83, 92, 94, 96, 98 and 98 %. Most ( $F_2$ ,  $F_3$ ,  $F_4$ ) plants in this cross were highly fertile but hybrid effects were disclosed in 2 sterile  $F_1$  individuals and in 3  $F_2$  and 2  $F_3$  plants that never flowered. In  $F_4$  on the other hand only one plant had reduced fertility and the seed set was unusually good (46.9-64.8 %).

*Ssp. argolica* x *spruneri*: Five population combinations gave 22  $F_1$  plants. Pollen stainability was > 94 % in 4 individuals; in all others it ranged from 41 to 76 %. In  $F_2$  plants the stainability varied from 0 to 88 %;  $F_3$  showed a similar range.

It is difficult to explain the reciprocal differences in pollen stainability between *spruneri* x *ssp. argolica* and *ssp. argolica* x *spruneri*. Chromosome counts and meiosis offered no clues either. Chromosomes were counted in 38 hybrids. All had  $2n=34$  and no polyploidy was found.

Chromosome pairing was good and meiosis essentially normal in  $F_1$ . *Ssp. argolica* x *spruneri* had some extra nucleoli in the tetrads and both combinations showed rare accessory nucleoli at diakinesis (*spruneri* x *ssp. argolica*) and at prophase II (*ssp. argolica* x *spruneri*). In  $F_2$  univalents were rare, laggards rare in *spruneri* x *ssp. argolica* and few in *ssp. argolica* x *spruneri*; the latter combination also had nucleoli in the tetrads. A few cells with accessory nucleoli occurred at prophase II in both combinations. It is difficult to draw any conclusions from these data since good pairing and few abnormalities were typical of meiosis in other crosses in the *swainsonii* complex.

A possible explanation for the high fertility in *spruneri* x *ssp. argolica*, cross no. 208 (mentioned above) through the fourth generation would be hybridization and introgression in the past involving *spruneri* (or ancestor). Some plants from the Dhrepanon area have *spruneri*-like characters and the *ssp. argolica* parent happened to be one of these. If hybridization has occurred, the genetic relationship between *spruneri* and *spruneri*-like plants would be close

and plants with high pollen fertility could be produced.

By contrast the pollen stainability was reduced in all  $F_1$  of sprunerii x ssp. swainsonii and reciprocal cross (Table 36).

Table 36. Percentage pollen stainability and seed set (*italics*) in sprunerii x ssp. swainsonii. - no flowers.

sprunerii x ssp. swainsonii

|       | No 204           | 205                         | 206                       | 722               | 1411                               |
|-------|------------------|-----------------------------|---------------------------|-------------------|------------------------------------|
| $F_1$ | 37 45<br>6.2<br> | 42 42 44 52<br>0 7.7 0<br>  | 34 31 42 46<br>0 17.5<br> | 22 38 42<br>0<br> | 44 46 47 48 75<br>21.9 4.3 2.2<br> |
| $F_2$ | 48<br>10.7<br>   | 47 53 62<br>0 12.5 12.5<br> | 51<br>                    |                   | 51 78 87<br>13.5 0<br>             |
| $F_3$ | 3<br>            | 86 99<br>                   |                           |                   | 67 94<br>32.1<br>                  |
| $F_4$ |                  |                             |                           |                   | 40 81 84 94 94<br>                 |

ssp. swainsonii x sprunerii

|       | No 316       | 318 | 319                           |
|-------|--------------|-----|-------------------------------|
| $F_1$ | 40 52<br>1.8 |     | 32 33 45 49 57<br>1.9 0 2.1 0 |

no  $F_2$  plants

Seed set in  $F_1$  hybrids

Flowers of the  $F_1$  hybrids were artificially self-pollinated (Table 37).

Well-formed nutlets were produced in 106 combinations, but the output was low and often very variable within combinations Table 38.

Table 37. Survey of number of flowers pollinated in the main crossing programme. The number of flowers are arranged in classes. The figures in the table are the number of hybrid combinations in  $F_1$  and  $F_2$  in each class. Details in the Appendix.

|                              | <10   |       | 10-19 |       | 20-49 |       | 50-99 |       | 100-199 |       | >200  |       | flow. |
|------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|---------|-------|-------|-------|-------|
|                              | $F_1$ | $F_2$ | $F_1$ | $F_2$ | $F_1$ | $F_2$ | $F_1$ | $F_2$ | $F_1$   | $F_2$ | $F_1$ | $F_2$ | poll. |
| ssp. swainsonii              | -     | 2     | -     | 1     | 2     | 1     | 2     | 1     | 6       | 1     | 1     | 1     |       |
| " scyronica                  | -     | -     | -     | 1     | 3     | 1     | 3     | 2     | 3       | 1     | -     | -     |       |
| " melangavica                | 1     | 2     | 1     | -     | -     | -     | 2     | 1     | 2       | 1     | -     | -     |       |
| " argolica                   | -     | -     | -     | -     | -     | 3     | 2     | 3     | 4       | 1     | 6     | 3     |       |
| spruneri                     | -     | -     | -     | -     | 1     | -     | 1     | 2     | -       | 1     | 3     | 1     |       |
| ionica                       | -     | 1     | -     | 2     | 4     | 3     | 3     | 1     | 4       | -     | -     | -     |       |
| euboica                      | 2     | 1     | -     | -     | 2     | 3     | 2     | 1     | 1       | 1     | 2     | -     |       |
| candida                      | -     | 2     | 1     | 2     | 2     | 4     | 4     | -     | 1       | 1     | 3     | -     |       |
| chrysantha                   | 2     | -     | -     | -     | 4     | 2     | -     | -     | 1       | -     | -     | -     |       |
| ssp. spreit.                 | -     | -     | -     | 1     | 2     | 2     | 3     | 3     | 3       | 2     | 4     | -     |       |
| " virella                    | -     | -     | 1     | 1     | 3     | 3     | 2     | 2     | 1       | -     | 3     | -     |       |
| messeniaca                   | -     | 3     | -     | -     | 5     | 2     | 1     | 1     | 4       | -     | 1     | -     |       |
| mollissima                   | 1     | -     | 2     | -     | -     | 1     | 3     | -     | 1       | -     | 2     | -     |       |
| total number of combinations | 6     | 11    | 5     | 8     | 28    | 25    | 28    | 17    | 31      | 9     | 25    | 5     |       |

Table 38. Seed set (%) in  $F_1$  hybrids. The values for pure taxa were obtained after self-pollination. - no plants available.

| female \ male    |                 |                |                  |               |          |        |         |         |            |              |              |           |            |
|------------------|-----------------|----------------|------------------|---------------|----------|--------|---------|---------|------------|--------------|--------------|-----------|------------|
|                  | ssp. swainsonii | ssp. scyronica | ssp. melangavica | ssp. argolica | spruneri | ionica | euboica | candida | chrysantha | ssp. spreit. | ssp. virella | messeniac | mollissima |
| ssp. swainsonii  | 50.0            | 17.6           | 11.5             | 1.0           | 1.2      | 0.8    | 0.8     | 5.0     | 0          | 0            | 0.2          | 0.7       | -          |
| ssp. scyronica   | 3.4             | 50.0           | 7.9              | 3.0           | 2.2      | 3.0    | 3.6     | 0.3     | 0          | 0            | 0            | -         | -          |
| ssp. melangavica | 2.3             | 3.6            | 87.5             | 9.8           | 1.9      | -      | 0       | -       | -          | -            | -            | -         | 12.5       |
| ssp. argolica    | 6.0             | 0.2            | 3.6              | 45.0          | 7.7      | 3.8    | 0.3     | 0.7     | 0.2        | 10.0         | 1.3          | 3.2       | 0          |
| spruneri         | 5.4             | 5.2            | 3.1              | 9.7           | 87.5     | -      | -       | 0       | -          | -            | -            | -         | -          |
| ionica           | 3.4             | 0.7            | -                | 0.3           | 2.7      | 77.5   | 1.8     | 2.9     | 5.6        | 0            | 3.9          | 15.2      | 9.3        |
| euboica          | 1.4             | 6.7            | 0.7              | 1.4           | 1.4      | 3.8    | 70.0    | 14.3    | 6.2        | -            | 1.6          | -         | -          |
| candida          | 5.4             | 1.7            | 4.7              | 2.3           | 1.0      | 7.1    | 12.3    | 57.5    | 34.4       | 5.8          | 2.8          | 6.6       | 1.5        |
| chrysantha       | 2.0             | 0              | -                | 1.1           | 1.1      | -      | 0.7     | 21.4    | 50.0       | 0.5          | 5.1          | 5.6       | -          |
| ssp. spreit.     | 0.2             | 10.9           | 0                | 0.9           | 3.0      | 2.2    | 0       | 11.3    | 11.5       | 70.0         | 26.6         | 2.8       | 1.2        |
| ssp. virella     | 0.4             | 0.4            | -                | 0             | -        | 1.9    | 2.2     | 3.8     | 15.6       | 14.0         | 50.0         | 5.0       | 0.7        |
| messeniac        | 2.4             | 1.4            | 0                | 0             | 2.2      | 11.0   | 1.1     | 6.2     | -          | 4.4          | 3.8          | 52.5      | 4.2        |
| mollissima       | 0.5             | -              | 0                | 1.8           | 1.8      | -      | 0       | -       | -          | -            | 0            | -         | 62.5       |



Ssp. swainsonii x ssp. scyronica and ssp. swainsonii x ssp. melangavica gave the best results in the swainsonii group and candida x chrysanthia and ssp. spreitzenhoferi x ssp. virella in the candida group. Some of the combinations that yielded no good seeds showed evidence of fertilization and sometimes even small shrivelled nutlets were formed. Such nutlets were tested but did not germinate and were not counted as yield. Seed set in relation to pollen stainability in the  $F_1$  hybrids is presented in Fig. 25. Pollen stainability in plants that yielded no nutlets ranged from 23% to 63% but hybrids that did set seeds also fell within this range. Most plants with 50%-76% pollen stainability produced a varying amount of nutlets (up to 15%). Highly pollen-fertile individuals (95-98%), which were very few, yielded 21.5%-34.5% nutlets.

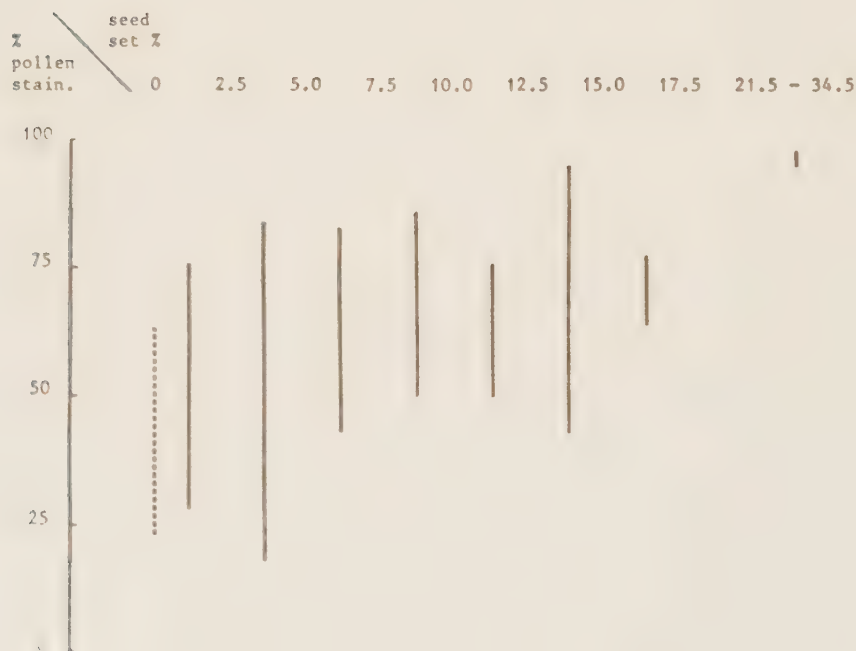


Fig. 25. Range of pollen stainability in  $F_1$  hybrids which yielded a certain amount of nutlets (% of maximum, 4 nutlets = 100%). The pollen stainability in plants which produced 0 nutlets ranged from 23 to 63 (.....).

### Germination of $F_1$ seeds

Germination of seeds, produced in  $F_1$  plants, was satisfactory in the *swainsonii* group but considerably lower in the *candida* group (Table 27). All nutlets in 20 combinations (of 106) failed to germinate -- 1 or 2 combinations in most taxa (except *spruneri*), 3 in *ssp. virella* and 4 in *chrysantha*. But the nutlets were few in the failing combinations.

### Morphology of the $F_2$ hybrids

Many of the  $F_2$  plants were vigorous and well developed but small plants were commoner and abnormalities more frequent than in  $F_1$ . The variation was much greater and sometimes spanned the entire range between the two parents. *Ssp. argolica* x *ssp. spreitzenhoferi* for instance displayed a typical  $F_2$  segregation. Occasionally one plant in a combination would flower abundantly e.g. *ssp. swainsonii* x *ssp. melangavica* (pollen fertility 99%, seed set 18.3%) and *ssp. virella* x *ssp. swainsonii* (51%, 7.7%, all flowers lacked the purplish-red pattern).

Sometimes a character in one or a few plants in a combination resembled that of a third taxon as for example the *ssp. scyronica*-like leaves of two hybrids of *ssp. argolica* x *ssp. melangavica* (more rounded than those of the parents). Adding the characters (inherited from *ssp. melangavica*) which *ssp. scyronica* and *ssp. melangavica* have in common, such as two-flowered verticillasters and very obtuse angle of the lower lip, one of these plants looked altogether *scyronica*-like, except for flower pattern.

Also, two plants of *spruneri* x *ssp. scyronica* resembled *ssp. melangavica*; the flower even lacked the upper lip marks -- this character segregated in  $F_2$ . These findings were intriguing and inspired to a separate crossing programme including three parents in each cross. See Triple crosses, p. 139.

The leaf shape and petiolate floral leaves (key characters in *ionica*) of two plants of *candida* x *ssp. swainsonii* and one of *candida* x *ssp. argolica* resembled

those of *ionica*.

Some of the *candida* x *messenica* plants were morphologically rather similar to the suspected natural hybrids. The results of pollen fertility and seed set suggest that hybrids most likely could establish themselves in nature. Pollen fertility was reduced in the suspected natural hybrids.

*Chrysanthus* x ssp. *virella* resembled natural hybrids on the Monemvasia peninsula. This will be reported on separately.

Finally, the hybrids of ssp. *swainsonii* x ssp. *argolica* and ssp. *argolica* x *spruneri* formed a bewildering array of plants, many resembling individuals in the field.

The morphology of the  $F_2$  hybrids seems to reveal a network of interrelated taxa.

#### Abnormalities in $F_2$

Although most hybrids were morphologically well developed abnormalities of one or more kinds appeared in c. 20% of the  $F_2$  hybrids.

Usually two normal reniform cotyledons developed. But irregularities occurred in about 4% of the seedlings some of which had one cotyledon, others three and still others two which deviated in size and shape (obcordated or peltate). The abnormal seedlings either died early, became stunted or developed normally. Chlorotic seedlings were few and died early.

About 2.5% produced only one or two flowers on the whole plant and as many hybrids developed no flowers at all the first year (four plants flowered the second season). Minute buds that never opened were common in  $F_2$  of *euboica* x ssp. *virella*. In three plants vegetative growth continued beyond the inflorescence.

Malformations of the corolla in some or all of the flowers of the plant appeared in about 5%. The upper lip was often split in two and/or the lower lip either irregularly lobed or extremely large. One peloric flower, at the top of

the inflorescence, was noted in *ssp. argolica* x *ssp. melangavica*. Colour deviations occurred in 3%: some flowers lacked the reddish-purplish pattern; others had a greenish upper lip, a greenish spot in the middle or a white-and-green variegated corolla (often in hybrids involving *ssp. scyronica* and *ssp. melangavica*). Regular-sized flowers with a short pistil (half size or less) were found in 1%.

In 1.5% some or all flowers were minute. Abortive development of the anthers and a short pistil (about half the normal size) were common in these flowers. Pollen stainability was 0% (rarely up to 2%). In one plant nutlets were produced after cross-pollination. These plants were gynomonoecious; there were regular, perfect flowers and female flowers on the same plant, often in the same inflorescence and even in the same verticillaster. The proportion between the two kinds of flowers varied with the individual. Taken over all generations these gynomonoecious hybrids mostly involved crosses of *ssp. spreitzenhoferi* and *ssp. virella*, (also *chrysantha* and *candida*) while deformed corollas and irregularities of the pistil were commoner in combinations of the *swainsonii*-group.

Besides degenerated anthers other abnormalities of the stamens occurred. In one *spruneri* x *ssp. argolica* filament and anthers of two stamens were connate in some flowers. In *mollissima* x *ssp. argolica*, the filaments had a continuation of corolla tissue, complete with a reddish pattern beyond the anthers which were sterile. In some *messenica* x *spruneri* plants, flowers with five or six stamens were found. The pistil had a trifurcate stigma and six ovules. Introrse dehiscence was recorded in a few flowers of *ssp. melangavica* x *ssp. scyronica*. In one flower of *ssp. argolica* x *ssp. virella* the entire filament was attached to the corolla so that the anther was appressed to the lower lip. Deviation in pollen size and a reduced amount of pollen was noted in some hybrids.

The normally bifurcate stigma was undivided in 1% of the  $F_2$  plants and the ovules were usually only two (occasionally three) in these flowers as in *ssp. argolica* x *ssp. swainsonii*, for instance. Some of these flowers were pollinated

but no nutlets were produced. In one *ssp. melangavica* x *ssp. scyronica* one nutlet germinated while still on the plant. The seedling was transferred to soil and grew into a normal individual.

Grant (1963) attributes failure to develop normal anthers in hybrids to the result of an unfavourable combination of genes. Sexual dimorphism has been found in several genera of the Lamiaceae i.e. *Origanum*, *Thymus*, *Nepeta*, *Glechoma* and *Salvia*. Bhattacharjee (1973) reported gynodioecism in *Stachys bysantina*, *S. germanica* and *S. cretica*. Wilcock and Jones (1974) found male-sterility and significantly smaller flowers than was usual in populations of *S. palustris*.

#### Pollen stainability in $F_2$ hybrids

The range of pollen stainability in  $F_2$  was even wider than in  $F_1$ , very low, medium and fairly high percentages occurring within the same combination (Fig. 26 and Table 33). Reciprocal differences were not pronounced except in *spruneri* x *ssp. argolica* (even more so in  $F_3$  and  $F_4$ ) and to a lesser degree in *euboica* crosses with the *swainsonii* subspecies. Occasionally  $F_1$  plants with low fertility gave rise to progeny with considerably higher fertility as in *candida* x *ssp. melangavica* ( $F_1$  43%, 8 plants,  $F_2$  85%, 2 plants). *Ssp. spreitzenhoferi* x *ssp. scyronica* showed another pattern. If at least one plant manages to pass the initial barriers the cross may be successful and produce fertile progeny. Superficially this may seem like a distorted picture of the relationship between the two species, but instances such as this may have been evolutionary significant since the *Stachys* species seem to form a network of interrelationships. Pollen stainability and seed set in *ssp. spreitzenhoferi* x *ssp. scyronica* are shown in Table 39. (The reciprocal cross *ssp. scyronica* x *ssp. spreitzenhoferi*: 11 plants, pollen stainability 29% (9--42%), 111 flowers were pollinated but no nutlets were produced.)



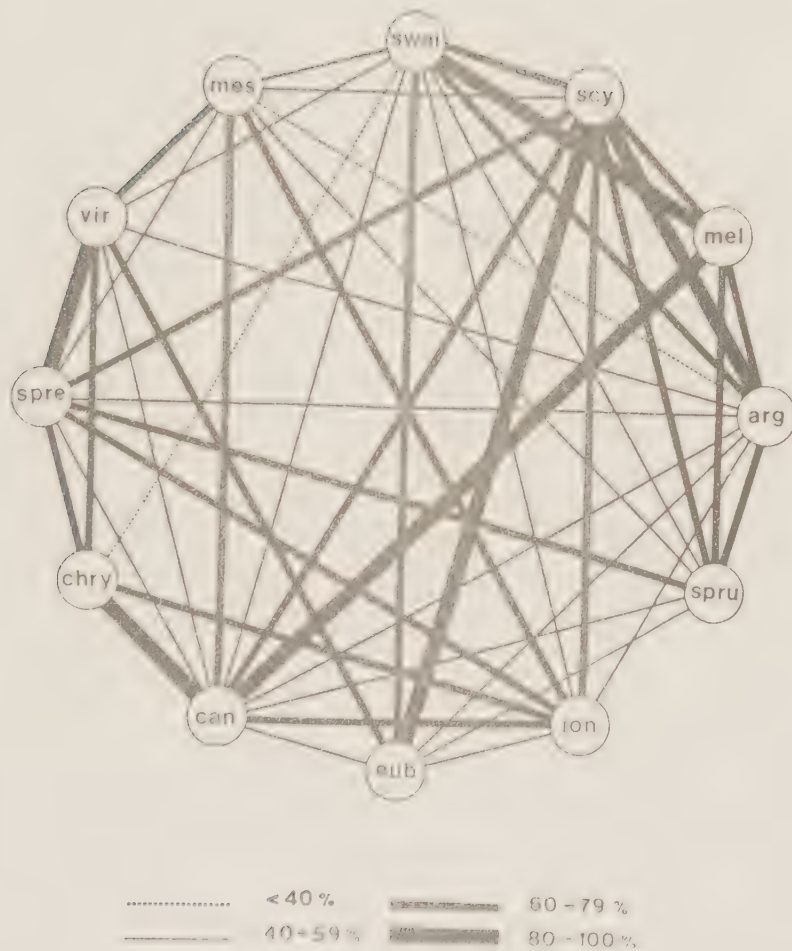


Fig. 26. Pollen stainability in  $F_2$ . Traits are indicated by the first letters of the specific and subspecific name. Each line represents the average of the mean values for all crosses between two traits.

Table 39. Percentage pollen stainability and seed set (*italics*) in ssp. spreitzenhoferi x ssp. scyronica. - no flowers.

|       |        |         |         |           |    |    |          |            |           |            |            |          |    |    |         |
|-------|--------|---------|---------|-----------|----|----|----------|------------|-----------|------------|------------|----------|----|----|---------|
|       | No 275 | No 276  |         |           |    |    |          |            |           |            |            |          |    |    |         |
| $F_1$ | 9<br>0 | 25<br>0 | 27      | 31<br>0   | 41 | 43 | 76<br>32 |            |           |            |            |          |    |    |         |
| $F_2$ |        |         | 26<br>0 | 46<br>9.1 | 69 | 78 | 80<br>49 | 91<br>42.9 | 94        | 95         | 96<br>3.4  |          |    |    |         |
| $F_3$ |        |         |         |           |    |    | 90       | 93<br>42.5 | 94<br>4.2 | 95<br>40.7 | 75<br>11.4 | 81<br>25 | 92 | 93 | 94<br>0 |

Seed set in F<sub>2</sub> hybrids

As in  $F_1$  seed set was mostly low and variable but nutlets were obtained in 59 combinations (Table 40). There was a certain correlation between seed set in  $F_1$  and  $F_2$  but also some exceptions to this. *Ssp. swainsonii* x *ssp. scyronica* and *ssp. swainsonii* x *ssp. melangavica* and *spruneri* x *ssp. argolica* were most successful in the *swainsonii* group and *candida* x *chrysantha* and *ssp. virella* x *ssp. spreitzenhoferi* in the *candida* group. Occasional hybrids between the two groups gave exceptionally high values such as *ionica* x *chrysantha* and *messenica* x *ionica* but only 5 and 6 flowers were pollinated in these combinations. In *ssp. spreitzenhoferi* x *ssp. scyronica* on the other hand 165 flowers were pollinated which gave 173 nutlets, but these plants were the progeny of the only successful  $F_1$  individual (of 7) in this cross (referred to in 'Pollen stainability in  $F_2$ ').

Table 40. Seed set (%) in  $F_2$ . - no plants available.

| female \ male    | ssp. swainsonii | ssp. scyronica | ssp. melangavica | ssp. argolica | spruneri | ionica | euboica | candida | chrysantha | ssp. spreit. | ssp. virella | messeniacia | mollissima |
|------------------|-----------------|----------------|------------------|---------------|----------|--------|---------|---------|------------|--------------|--------------|-------------|------------|
| ssp. swainsonii  |                 | 19.5           | 11.1             | 0.7           | -        | 10.0   | 0       | 7.8     | -          | -            | -            | 7.1         | -          |
| ssp. scyronica   | 1.9             |                | 2.3              | 0             | -        | 4.5    | 2.9     | -       | -          | -            | -            | -           | -          |
| ssp. melangavica | 5.6             | 2.7            |                  | 8.5           | 10.0     | -      | -       | -       | -          | -            | -            | -           | -          |
| ssp. argolica    | 9.4             | 1.6            | 2.9              |               | 6.9      | 6.2    | 4.5     | 0       | -          | 13.4         | 1.1          | 2.6         | -          |
| spruneri         | 7.4             | 4.8            | 8.5              | 11.8          |          | -      | -       | -       | -          | -            | -            | -           | -          |
| ionica           | 8.9             | 3.8            | -                | -             | -        |        | 0       | 0       | 60.0       | -            | -            | 6.8         | 7.5        |
| euboica          | 0               | 0.7            | -                | 2.2           | 0        | 0.5    |         | -       | -          | -            | 0            | -           | -          |
| candida          | 11.5            | 2.3            | 0                | 11.4          | 0        | -      | 0       |         | 21.4       | 0            | -            | 3.4         | -          |
| chrysantha       | -               | -              | -                | -             | -        | -      | -       | 20.4    |            | -            | 0            | -           | -          |
| ssp. spreit.     | -               | 26.2           | -                | 4.4           | 9.5      | 5.6    | -       | 14.5    | 5.9        |              | 14.9         | 6.0         | -          |
| ssp. virella     | 9.4             | -              | -                | -             | -        | -      | 4.5     | 0       | 1.7        | 26.0         |              | 1.0         | -          |
| messeniacia      | -               | 0              | -                | -             | 7.7      | 25.0   | -       | 0       | -          | 9.5          | 12.5         |             | -          |
| mollissima       | -               | -              | -                | 5.0           | -        | -      | -       | -       | -          | -            | -            | -           | -          |

All hybrids were artificially self-pollinated. For comparison a few hybrids were cross-pollinated within families (family = progeny of a single  $F_1$  plant). Three families had higher seed set, another three had lower and five had the same result, zero, after both self- and cross-pollination. Since this method could not be used for all plants at all times self-pollination was preferred. Cross-pollination may not have given a more reliable picture because the pollen stainability within a family often varied widely.

Later generations

$F_3$  hybrids

The number of combinations as well as the number of plants were much reduced in  $F_3$ .

Germination. The  $F_2$  nutlets germinated poorly except for those set by hybrids involving *euboica* (Table 27).  $F_3$  plants were raised in 37 combinations. In addition to the reduced and variable germination of previous generations there seems to be further failure to germinate, probably a manifestation of hybrid breakdown.

Morphology. The morphological variation kept within the range of that in  $F_1$  and  $F_2$  but the difference in vigour within combinations was pronounced. The same kind of abnormalities as in earlier generations appeared. Some plants bore only a few flowers. In several combinations where flowering was sparse or normal an occasional individual flowered extremely abundantly, e.g. *ssp. argolica* x *ssp. melangavica*. In one *ssp. argolica* x *ssp. spreitzenhoferi* plant minute flowers which never opened were hidden deep down in the calyx.

Pollen stainability. Most plants were semifertile even though the range of stainability was wide in many combinations, Fig. 27.

Seed set. Nutlets were produced in 24 combinations and in general the percentage was higher than in earlier generations. By contrast *candida* x *chrysantha* did not yield any seeds in spite of high pollen fertility, and good seed set and high pollen fertility in previous generations. This fact together with poor germination and generally weak plants in  $F_3$  suggest the presence of definite barriers between the two species even though they did not appear till late.

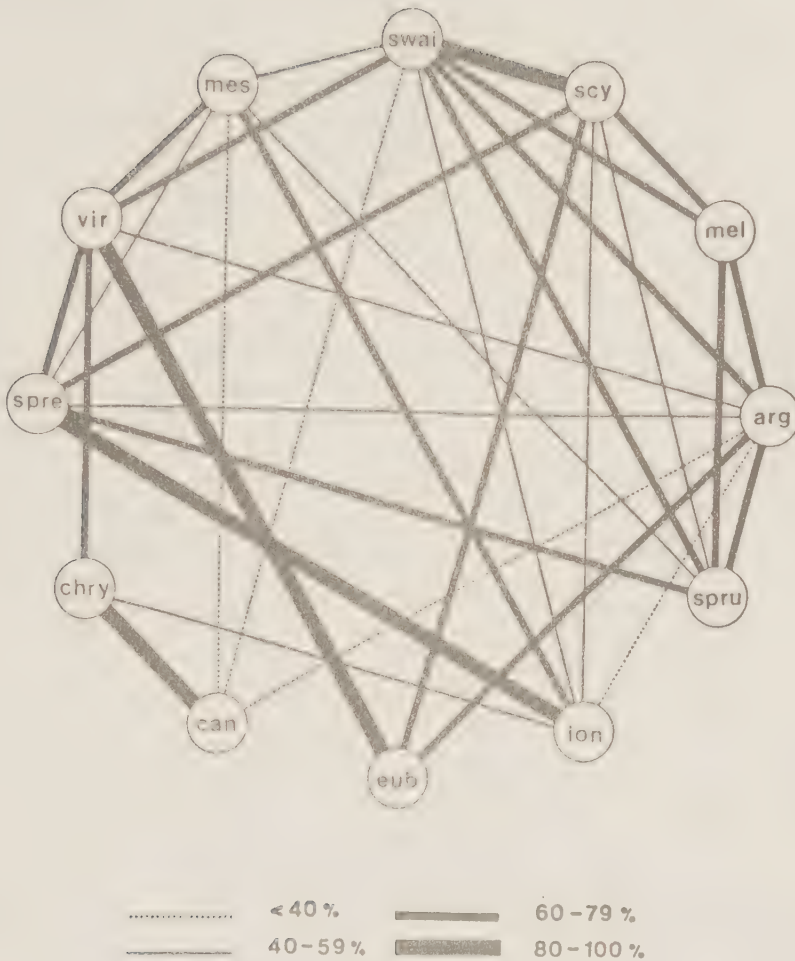


Fig. 27. Pollen stainability in  $F_3$ . Taxa are indicated by the first letters of the specific and subspecific name. Each line represents the average of the mean values for all crosses between two taxa.

#### $F_4$ hybrids

A few combinations continued till the fourth generation. Fig. 28 shows the pollen stainability. At this stage the swainsonii and the candida groups had lost all direct connections but seemed to be linked by ionica through ssp. argolica and ssp. spreitzenhoferi. However, there were only 3 plants in the ssp. spreitzenhoferi x ionica cross and 5 in the ssp. argolica x ionica so unfortunately the link can only be speculated on.



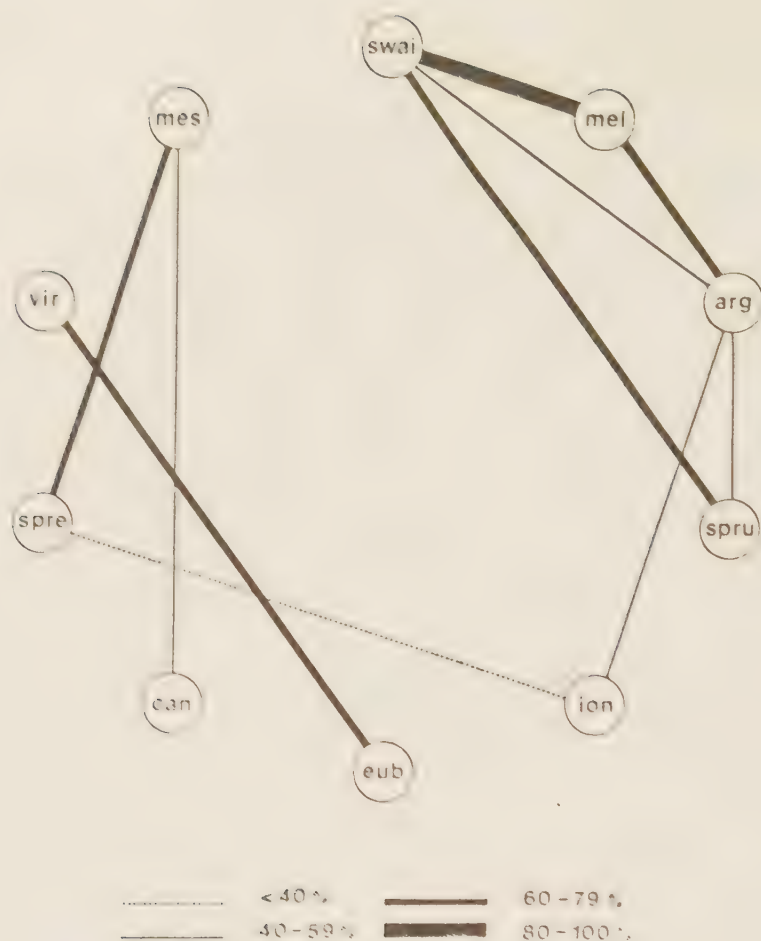


Fig. 28. Pollen stainability in  $F_4$ . Taxa are indicated by the first letters of the specific and subspecific name. Each line represents the average of the mean values for all crosses between two taxa.

One plant of *ssp. virella* x *euboica* survived. After cross-pollination this cross had exceptionally good seed set but it decreased rapidly in later generations. The range of pollen stainability (before  $F_4$ ) was wide but occasional plants were highly fertile. Morphologically these two species are clearly distinct but they have one feature in common -- numerous glands. They may be related chemically even though the odour is different.

A few of the other combinations comprised more plants; *spruneri* x *ssp.*

argolica 17 plants; ssp. spreitzenhoferi x ssp. virella 11; ssp. argolica x ssp. swainsonii 8, but all others had only 5 or less and the results must consequently be interpreted with caution.

#### Backcrosses

A few hybrids were backcrossed. Backcrosses of candida x chrysantha to both parents gave good pollen stainability (43, 56, 84, 85, 86 + 23 plants 90-99%) and 12% seed set in  $B_1$ ; 88-99% and 0% respectively in  $B_2$ . Messeniaca x candida gave 72-96% stainability and 36.4% seed set in  $B_1$ ; 0-83% and 14.9% in  $B_2$ .

#### Tetragona crosses

Tetragona was used as female parent in crosses with ssp. swainsonii, ssp. argolica, sprunerii, ionica and euboica and as male parent in crosses with the above except sprunerii and also with messeniaca, ssp. spreitzenhoferi and mollissima. The nutlets of the last two crosses did not germinate and the sprunerii cross yielded no nutlets. Otherwise the hybrid seeds germinated well and some  $F_1$  were raised. Most plants were morphologically intermediate but the long internodes and distant verticillasters of tetragona were very conspicuous in  $F_1$  as well as in  $F_2$ .

The pollen stainability in euboica and messeniaca hybrids was high in  $F_1$  (90% and 91%; in  $F_2$  80% and 74%) while other plants were semi-fertile. Reciprocal crosses of ionica, euboica and messeniaca gave the highest values (17.8%, 18.8%, 20.7%); otherwise the seed set was mostly low. Tetragona x ssp. argolica, ionica x tetragona and messeniaca x tetragona were the only three that produced nutlets in  $F_2$  (4.8%, 14.9%, 22.9%).

Triple crosses -- an attempt to find the origin of ssp. melangavica

The morphological characters of ssp. melangavica appear to be a combination of features of ssp. swainsonii, ssp. scyronica, ssp. argolica and sprunerii. Arti-

ficially produced hybrids ( $F_1$ ,  $F_2$  and  $F_3$ ) of two taxa in various combinations of these four sometimes resembled the wild *melangavica* plants but a few important characters were always lacking -- characters which a third taxon probably could provide. A crossing programme involving three taxa in each cross was therefore designed to test the hypothesis that *ssp. melangavica* has arisen through hybridization between some of these four taxa or their ancestors. The key characters of *melangavica* that the synthetic hybrid must possess were a small corolla, lack of purple marks on upper lip, angle of lower lip about  $150-168^\circ$ , short pistil, 2-flowered verticillasters, comparatively long and narrow calyx teeth and shape of floral and cauline leaves and indumentum as in *ssp. melangavica*.

Since two subspecies (various combinations of two of *ssp. swainsonii*, *ssp. scyronica* and *ssp. argolica*) and one species (*spruneri*) are involved in each cross, the term trispecific is not entirely appropriate. Therefore the words 'triple cross' and 'triple hybrid' are used to denote these combinations. The only trisubspecific combination is thereby also included.

Primary  $F_1$  hybrids (of two taxa) were used as female parents and plants of pure taxa as male parents. This proved to be more successful than crossing in the other direction, probably owing to the lower pollen fertility of the hybrids. The following combination of triple crosses were obtained (number of crosses in parentheses):

(*ssp. argolica* x *ssp. swainsonii*) x *spruneri* (6)

(*spruneri* x *ssp. swainsonii*) x *ssp. argolica* (4)

(*spruneri* x *ssp. argolica*) x *ssp. swainsonii* (2)

(*ssp. swainsonii* x *spruneri*) x *ssp. argolica* (1)

(*spruneri* x *ssp. swainsonii*) x *ssp. scyronica* (1)

(*ssp. argolica* x *ssp. swainsonii*) x *ssp. scyronica* (1)

(*ssp. scyronica* x *ssp. swainsonii*) x *spruneri* (1)

(*spruneri* x *ssp. swainsonii*) x *ssp. scyronica* (2)

(*ssp. swainsonii* x *ssp. scyronica*) x *spruneri* (3)

(*ssp. scyronica* x *spruneri*) x *ssp. swainsonii* (1)

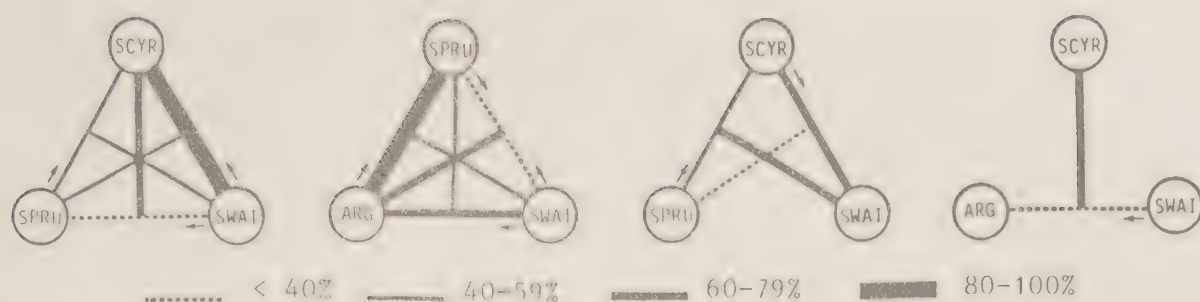


Fig. 29. Lines connecting two taxa (circles) represent pollen stainability in the primary hybrid. Lines perpendicular to them and leading to a third taxon represent pollen stainability in the triple hybrid. Arrows indicate direction of pollen transfer in the primary hybrid.

Nutlets were produced in most triple crosses ranging from 5 to 82%. Germination was satisfactory (25-100%) and 107  $F_1$  hybrids were raised. Pollen stainability (range 34-92%) is illustrated in Fig. 29. Fifty-eight plants were artificially selfed and yielded 2-19% nutlets, the cross (sprunerii x ssp. swainsonii) x ssp. scyronica having the highest seed set.

Germination ranged from 0 to 100% resulting in 51  $F_2$  triple hybrids. Pollen stainability (%):

|                                                | mean | range | no. of plants |
|------------------------------------------------|------|-------|---------------|
| (ssp. argolica x ssp. swainsonii) x sprunerii  | 84   | 82,86 | 3             |
| (sprunerii x ssp. swainsonii) x ssp. argolica  | 74   | 17-97 | 21            |
| (sprunerii x ssp. argolica) x ssp. swainsonii  | 91   | 84-99 | 4             |
| (ssp. swainsonii x sprunerii) x ssp. argolica  | 70   | —     | 1             |
| (sprunerii x ssp. swainsonii) x ssp. scyronica | 75   | 35-98 | 22            |

### Morphology

The triple hybrids displayed features of all three parents. There was some variation among progeny of the same cross and also among different crosses within



the same combination, particularly in shape of floral leaves and cauline leaves, type of leaf margin and shape of calyx teeth. Colour and pattern of the corolla also differed conspicuously but all hybrids of (*spruneri* x *ssp. swainsonii*) x *ssp. argolica* and (*spruneri* x *ssp. argolica*) x *ssp. swainsonii* inherited the typical *spruneri* colour (13D8).

Some of these hybrids resembled the confusing types on the Argolis peninsula.

*Ssp. melangavica* has some characters in common with *ssp. scyronica* but it lacks the purple nectar guides on the upper lip of the corolla, typical of *ssp. scyronica* (also lacking in *ssp. swainsonii*, *ssp. argolica* and *spruneri*). All  $F_1$  hybrids inherited these markings when *ssp. scyronica* was used as male parent in the triple cross, but some hybrids lacked this character when *ssp. scyronica* was a component of the primary hybrid (Table 41). This character segregated in  $F_2$ , for instance in (*spruneri* x *ssp. swainsonii*) x *ssp. scyronica* as illustrated in Table 42 (5 of 10  $F_1$  plants produced  $F_2$  progeny).

The  $F_1$  hybrids of this cross were more like *ssp. melangavica* than were the progeny of any other triple cross -- but all had the purple marks. In  $F_2$ , however, the individuals that lacked these marks greatly resembled *melangavica* in other characters as well. Two plants had 6-flowered verticillasters but such plants are also found occasionally in natural populations. The  $F_2$  were selfed resulting in 24% seed set. Thirteen  $F_3$  plants were raised but one was dwarfed and two died early. Progeny of plants with upper lip markings inherited these markings and progeny of plants without markings lacked them.

Table 43 shows pollen stainability and seed set in the cross (*spruneri* x *ssp. swainsonii*) x *ssp. scyronica*.



Table 41. Inheritance of upper lip markings (%) in triple crosses involving *ssp. scyronica*,  $F_1$ .

|                                                                           | %   | No. of<br>plants<br>in comb. |
|---------------------------------------------------------------------------|-----|------------------------------|
| ( <i>ssp. argolica</i> x <i>ssp. swainsonii</i> ) x <i>ssp. scyronica</i> | 100 | 6                            |
| ( <i>spruneri</i> x <i>ssp. swainsonii</i> ) x <i>ssp. scyronica</i>      | 100 | 2                            |
| ( " " " ) " "                                                             | 100 | 7                            |
| ( " " " ) " "                                                             | 100 | 10                           |
| ( <i>ssp. swainsonii</i> x <i>ssp. scyronica</i> ) x <i>spruneri</i>      | 25  | 4                            |
| ( " " " " ) "                                                             | 0   | 5                            |
| ( " " " " ) "                                                             | 33  | 6                            |
| ( <i>ssp. scyronica</i> x <i>ssp. swainsonii</i> ) x <i>spruneri</i>      | 75  | 8                            |
| ( <i>ssp. scyronica</i> x <i>spruneri</i> ) x <i>ssp. swainsonii</i>      | 0   | 2                            |

Table 42. Inheritance of upper lip markings in the triple cross (*spruneri* x *ssp. swainsonii*) x *ssp. scyronica* (no. 814).

+ = plants with markings, - = plants without markings.

|       | 814-01 | -02 | -05 | -08 | -10 |
|-------|--------|-----|-----|-----|-----|
| $F_1$ | +      | +   | +   | +   | +   |
| $F_2$ | +      | +   | +   | +   | +   |
| $F_3$ | +      | +   | +   | +   | +   |

Table 43. Percentage pollen stainability and seed set (*italics*) in the triple cross (*spruneri* x *ssp. swainsonii*) x *ssp. scyronica*. Seed set after pollination by *ssp. melangavica* in parenthesis. - no flowers. \* plant died early.

|                |                                        |                      |             |                                   |                                                                       |    |    |    |    |    |
|----------------|----------------------------------------|----------------------|-------------|-----------------------------------|-----------------------------------------------------------------------|----|----|----|----|----|
| F <sub>1</sub> | 39                                     | 46                   | 60          | 67                                | 71                                                                    | 72 | 84 | 92 | 93 | 98 |
| F <sub>2</sub> | 35 35 74 83<br>1.2<br>(x mel.<br>31.2) | 65 21                | 45 69 81    | 92 94<br>28.1<br>(x mel.<br>40.6) | 48 73 74 82 89 92 94 96 98<br>0.8 0 3.3 0 5<br>(x mel. 50) (x mel. 0) |    |    |    |    |    |
| F <sub>3</sub> | 19 78                                  | 83 80 72 86 87<br>20 | 65 77 90 92 |                                   |                                                                       |    |    |    |    |    |

The *melangavica*-like F<sub>2</sub> hybrids were crossed with *ssp. melangavica* yielding 5 plants. These individuals now comprised four taxa. They were vigorous and could hardly be distinguished from natural *ssp. melangavica* plants. Pollen stainability was 53, 73, 77, 82 and 96%.

Although the triple hybrids in this experiment are comparatively few (171) they reveal that these four taxa, *spruneri* and *ssp. swainsonii*, *ssp. argolica* and *ssp. melangavica* have a great potential to hybridize and produce fertile progeny. This is an important fact when hybridization is considered in evolutionary perspective.

Comments -- all crosses

At first glance germination and nutlet production may seem discouragingly low, but poor germination and low seed set have been reported for other *Stachys* species also. Bihlmeier (1928) found that seeds of *S. silvatica* germinated only after scarification -- in light 20%, in darkness 10%. *S. annua* on the other hand germinated without pretreatment -- in light 40%, in darkness 29.5%, at 28°. Pinfield et al. (1975), studying hormonal changes during after-ripening in seeds of *S. alpina*, found that the seeds germinated well when incubated in gibberellin. Chilling (5°) under moist conditions for 3-5 months resulted in

20-30% germination. Lang (1940), who performed extensive crossing experiments in several sections of *Stachys*, also experienced difficulties with germination, particularly with hybrid seeds. He too pollinated hundreds of flowers sometimes without getting a single nutlet, or just a few. Wilcock and Jones (1974) obtained 42% nutlets after self-pollination of *S. silvatica* and 26% after cross-pollination. The corresponding figures for *S. palustris* were 17% and 47.5%. *S. annua* is annual and the higher germination percentage could therefore be expected since high seed set and good germination are of the utmost importance for annuals. In perennials on the other hand these factors are generally less critical and lower values are usually found. However, additional factors evidently also lie behind the fairly low germination not only in the taxa used in this investigation but in other *Stachys* species as well and should be studied further.

Seen against this background the quantitative results for the *swainsonii* and *candida* groups are not surprising.

#### Meiosis in the hybrids

Satisfactory preparations of various stages were obtained in 88 combinations. Unfortunately all stages were not available in every combination and the number of plants in each combination was limited; the results should therefore be interpreted only as general trends. Meiosis was examined in some of the parents and found to be normal.

#### Results

Most cells were meiotically regular. The degree of pairing was extremely high and 17 bivalents were usually formed. But some abnormalities were also found. These are summarized for the *swainsonii* group + *euboica*, for the *candida* group and for hybrids between the two categories in Table 44 and for all combinations according to meiotic stages in Table 45. The frequency of abnormal cells is expressed: rare, 2% or less; few, 3--6%; moderate, 7--15%; common >15%. The *swainsonii* group + *euboica*. The intersubspecific hybrids as well as the

Table 44. Summary of abnormalities in meiosis in the hybrids within the swainsonii group plus euboica, within the candida group and between these two categories.

Comb = number of combinations where a particular stage was found.

R = rare, 2% or less; F = few, 3--6%; M = moderate, 7--15%; C = common

> 15%. The number of combinations where abnormalities were found are listed under R, F, M, and C.

#### Frequency of abnormal cells in the hybrids

|                                          | Stage | within the<br>swain. group<br>+ euboica |   |   |   |   | within the<br>candida<br>group |   |   |   |   | between the<br>two groups |   |   |   |   |
|------------------------------------------|-------|-----------------------------------------|---|---|---|---|--------------------------------|---|---|---|---|---------------------------|---|---|---|---|
|                                          |       | Comb.                                   | R | F | M | C | Comb.                          | R | F | M | C | Comb.                     | R | F | M | C |
| Univalents                               | M I   | 16                                      | 5 | 3 | 2 |   | 6                              | 2 | 2 |   |   | 14                        | 5 | 3 |   |   |
| Multivalents                             | -     |                                         |   |   |   |   |                                |   |   |   |   |                           |   |   |   |   |
| Laggards                                 | A I   | 16                                      | 5 | 6 | 2 |   | 6                              |   |   | 2 |   | 13                        | 5 | 3 | 1 |   |
|                                          | T I   | 12                                      | 2 | 2 |   |   | 5                              | 1 | 2 |   |   | 14                        | 1 | 1 |   |   |
|                                          | M II  | 16                                      | 7 | 3 | 1 |   | 6                              |   | 3 | 1 |   | 14                        | 4 | 4 | 1 |   |
|                                          | A II  | 11                                      | 2 | 2 |   |   | 5                              |   |   |   |   | 9                         | 3 |   |   | 1 |
|                                          | T II  | 12                                      | 2 | 2 |   |   | 3                              |   |   |   |   | 11                        | 3 | 2 |   |   |
| Bridges                                  | A I   | 16                                      | 3 |   |   |   | 6                              | 2 |   |   |   | 13                        | 3 |   |   |   |
|                                          | A II  | 11                                      | 1 |   |   |   | 5                              | 2 |   |   |   | 9                         | 1 |   | 1 | 1 |
|                                          | T II  | 12                                      | 1 |   |   |   | 3                              |   |   |   |   | 11                        |   |   |   |   |
| Micronuclei                              | T II  | 12                                      |   |   |   |   | 3                              | 1 |   |   |   | 11                        | 1 |   |   |   |
|                                          | Tetr  | 16                                      | 3 | 2 |   |   | 6                              | 1 | 2 | 1 |   | 15                        | 4 | 2 | 1 | 1 |
| Triads                                   | Tetr  | 16                                      |   |   |   |   | 6                              | 1 |   |   |   | 15                        |   |   |   |   |
| Irregular<br>segregation                 | M II  | 16                                      | 1 |   |   |   |                                |   |   |   |   |                           |   |   |   |   |
| ---                                      |       |                                         |   |   |   |   |                                |   |   |   |   |                           |   |   |   |   |
| Accessory<br>nucleoli                    | Diak  | 16                                      |   | 2 | 1 |   | 6                              | 1 |   |   |   | 15                        |   |   |   |   |
|                                          | P II  | 16                                      | 1 | 5 | 2 | 3 | 6                              |   | 3 |   |   | 13                        | 3 | 6 |   |   |
| Nucleoli<br>in the<br>cytoplasm          | T I   | 12                                      |   | 3 |   | 3 | 5                              |   |   |   | 1 | 14                        | 1 | 1 |   |   |
|                                          | P II  | 16                                      | 1 |   |   |   | 6                              |   |   |   | 1 | 13                        | 1 |   |   |   |
|                                          | A II  | 11                                      |   | 1 |   |   | 5                              |   |   |   |   | 9                         |   |   |   |   |
|                                          | T II  | 12                                      | 2 |   | 2 | 3 | 3                              |   |   |   |   | 11                        |   | 2 | 2 | 1 |
|                                          | Tetr  | 16                                      | 3 | 1 | 3 | 4 | 6                              |   | 1 |   |   | 15                        | 1 | 4 | 1 | 3 |
| Additional<br>nucleoli in<br>the nucleus | Tetr  | 16                                      | 1 | 2 | 5 | 2 | 6                              |   |   | 1 | 1 | 15                        | 2 | 3 | 1 | 1 |

Table 45. Occurrence of abnormalities (in addition to normal cells) at each stage of meiosis. The results from all crosses in  $F_1$ ,  $F_2$  and  $F_3$  where available are summarized for each stage. In the fractions the numerator expresses the number of combinations where abnormalities occurred and the denominator the number of combinations where a particular stage was found.

All combinations (mollissima and messeniaca included).

Frequencies denoted as in Table 44.

|              |                                       |       | Rare | Few | Mod | Common |
|--------------|---------------------------------------|-------|------|-----|-----|--------|
| Diakinesis   | accessory nucleoli                    | 6/46  | 2    | 3   | 1   |        |
| Metaphase I  | univalents                            | 27/47 | 13   | 12  | 2   |        |
|              | quadrivalents                         | 1/47  |      | 1   |     |        |
| Anaphase I   | laggards                              | 28/46 | 9    | 12  | 6   | 1      |
|              | bridges                               | 10/46 | 10   |     |     |        |
|              | irregular segregation<br>(16+18)      | 1/46  | 1    |     |     |        |
| Telophase I  | laggards                              | 11/40 | 6    | 5   |     |        |
|              | nucleoli in the cytoplasm             | 9/40  | 1    | 4   |     | 4      |
| Prophase II  | laggards                              | 1/44  | 1    |     |     |        |
|              | micronuclei                           | 1/44  | 1    |     |     |        |
|              | accessory nucleoli                    | 26/44 | 4    | 17  | 2   | 3      |
|              | nucleoli in the cytoplasm             | 4/44  | 2    |     |     | 2      |
| Metaphase II | laggards                              | 30/44 | 14   | 12  | 4   |        |
|              | nucleoli in the cytoplasm             | 1/44  |      | 1   |     |        |
|              | irregular segregation<br>(16+18)      | 1/44  | 1    |     |     |        |
| Anaphase II  | laggards                              | 11/31 | 5    | 5   |     | 1      |
|              | bridges                               | 6/31  | 4    |     | 1   | 1      |
|              | nucleoli in the cytoplasm             | 1/31  |      | 1   |     |        |
| Telophase II | laggards                              | 13/34 | 6    | 7   |     |        |
|              | bridges                               | 3/34  | 2    | 1   |     |        |
|              | micronuclei                           | 4/34  | 2    | 1   |     | 1      |
|              | nucleoli in the cytoplasm             | 16/34 | 2    | 4   | 6   | 4      |
| Tetrad stage | micronuclei                           | 20/46 | 8    | 7   | 3   | 2      |
|              | nucleoli in the cytoplasm             | 25/46 | 5    | 7   | 6   | 7      |
|              | additional nucleoli in<br>some nuclei | 22/46 | 3    | 7   | 8   | 4      |
|              | triads                                | 1/46  | 1    |     |     |        |



spruneri hybrids gave similar results with mostly rare or few deviations. Ionica and euboica hybrids showed the same type of abnormalities but univalents (ssp. argolica x ionica and ssp. scyronica x euboica) and laggards (ssp. argolica x ionica and ssp. scyronica x ionica) were somewhat more frequent.

The  $F_1$  hybrid of ssp. scyronica x spruneri also showed a moderate number of laggards (rare in  $F_2$ ) and ssp. swainsonii x ssp. scyronica  $F_2$ , a moderate number of micronuclei. In addition irregular segregation (16+18) was observed at anaphase I and metaphase II in a ssp. scyronica x euboica cross. Abnormal nucleolar behaviour was noted such as accessory nucleoli at diakinesis and prophase II, extra nucleoli in tetrad nuclei and nucleoli of various sizes in the cytoplasm.

The candida group. Abnormal cells were rare or few in most combinations. In the cross chrysantha x ssp. virella however, the frequency of laggards was increased at anaphase I and metaphase II. Micronuclei, additional nucleoli in the tetrad nuclei and nucleoli in the cytoplasm were also seen oftener than in other crosses. Rare triads were present in one hybrid of candida x ssp. virella.

The swainsonii group + euboica x the candida group. In general, results of meiosis in hybrids between the two groups and in hybrids within each of the groups were very similar. There were some exceptions however, mainly spreitzenhoferi combinations. Thus laggards were moderately frequent and bridges common at anaphase II in ssp. scyronica x ssp. spreitzenhoferi. Bridges were also common in the ssp. argolica x ssp. spreitzenhoferi hybrids, which furthermore displayed many micronuclei. Nucleoli in the cytoplasm were frequent in these two combinations as well as in ssp. swainsonii x candida and ionica x ssp. spreitzenhoferi. The latter cross had additional nucleoli in the nucleus of many tetrads, as did the ssp. spreitzenhoferi x ssp. melangavica hybrids.

Messeniaca hybrids. Few univalents, few laggards (moderate in ssp. spreitzenhoferi x messeniaca), rare bridges, few extra nucleoli and a few quadrivalents (at metaphase I in candida x messeniaca) were recorded in hybrids with the candida

group, excepting *chrysantha*. Crosses with *ssp. swainsonii* and *ssp. argolica*, gave similar results except for bridges and quadrivalents which were not found.

Mollissima hybrids. Hybrids between *mollissima* and *ssp. melangavica*, *ssp. argolica* and *sprunerii* showed occasional laggards and micronuclei and hybrids with *ionica*, a few univalents.

Triple hybrids. Preparations were obtained from 11 triple hybrids. Even in these chromosome pairing was regular. Laggards were few at metaphase I, anaphase I and metaphase II; telophase II and the tetrad stage appeared to be normal except for occasional nucleoli in the cytoplasm. Some individuals had superfluous nucleoli at prophase II.

Nucleoli. Cells with a deviating number of nucleoli were observed at some meiotic stages in the hybrids (rarely in the 'pure' taxa). They occurred in many hybrid combinations but were usually rare or few in each combination. Normal cells have a single nucleolus at diplotene and diakinesis, but two nucleoli were occasionally noted. At prophase II (3--4(--7), (2+1, 2+2, 2+3, 2+4, 3+4, instead of 1+1) were sometimes seen.

At the tetrad stage some of the nuclei had 2 or 3 nucleoli instead of only 1. When 2 were present they were usually of a similar size, but otherwise they varied. At telophase II and at the tetrad stage nucleoli of different sizes also appeared in the cytoplasm.

Table 44 shows the frequency of cells with additional nucleoli. The increase in number of nucleoli is somewhat difficult to interpret. All chromosomes are small and similar and the karyotypes of all taxa are alike, so that it was not possible to distinguish any particular chromosome carrying the nucleolar organizing region, or to detect any structural changes such as translocations, inversions, duplications or deletions. Normally one nucleolus is present at diakinesis and often more than one chromosome seems to be associated with it.

Various causes of increased number of nucleoli have been suggested. Levin

(1973) found accessory nucleoli at diplotene and diakinesis in microsporocytes of natural hybrids between *Phlox pilosa* ssp. *pilosa* and ssp. *fulgida*. Although chromosome pairing was fairly normal, a large number of plants had cells with accessory nucleoli. There was a striking correlation between population hybridity and incidence of accessory nucleoli ( $r = 0.80$ ). Levin concluded that the accessory nucleoli in *Phlox* are a manifestation of multiple nucleolar organizers "which are normally latent but are activated by some perturbation related to hybridity".

Structural changes involving the nucleous organizing region (NOR) could presumably affect the pattern of nucleolus formation. Breaks within the NOR and translocation of a segment to a chromosome without a NOR could result in an increase in nucleolar number. Nicoloff et al. (1977) reporting on root-tip mitosis in *Hordeum vulgare* described such translocations, which gave rise to additional nucleoli. Breaks in the NORs have been reported in several other species but nucleolar behaviour was not mentioned. If more than one primary nucleolus is normally organized then they probably fuse immediately, since only one nucleolus is seen at prophase I. If in fact several nucleoli are formed, then malfunction or inhibition of the process governing the nucleolar fusion could cause the nucleoli to remain separate.

Whatever the cause the modification of the normal pattern of nucleolus organization in the hybrids reveals disharmony between the parental genomes.

#### Relations between pollen stainability and meiosis

Chromosome pairing was usually normal regardless of percentage of pollen stainability.

Univalents, rare or few, occurred in hybrids with a pollen stainability from 18% to 89% (no preparations were available from plants with lower %). Only 2 combinations had moderate univalents, ssp. *scyronica* x *euboica* and ssp. *argolica* x *ionica* with 66% and 42%.

Laggards, rare or few, were mostly found where pollen stainability was below 64%, and in two crosses, though rarely (*candida* x *chrysantha* and *ssp. spreitzenhoferi* x *ssp. virella*) in spite of high fertility (95--98%). In plants with more laggards, the stainability was lower than 59% (except in one plant, 64%) but hybrids without laggards also fell within the same range. Bridges were common in one combination only, *ssp. argolica* x *ssp. spreitzenhoferi* (and reciprocal) with 48% and 57% stainability.

Thus frequency and type of meiotic abnormalities cannot be directly correlated with reduced pollen stainability. In summary, univalents, laggards, bridges and micronuclei were rare or few in most of the hybrid combinations where they occurred except in the crosses mentioned.

The results implied closely related genomes without major structural differences between taxa in *S. swainsonii* and *sprunerii*. The disharmony is probably caused by minor structural differences. *Euboica* and *ionica* and the rest of the species used in the crossing programme seem to be more distantly related. There was some evidence of differences in the *candida* group between *candida* and *chrysantha* on the one hand and the subspecies of *spreitzenhoferi* on the other.

## CONCLUSIONS

Conclusions from the crossing experiments can be summarized as follows.

- All taxa are self-compatible but not spontaneously self-fertilizing.
- Most taxa were readily crossed -- 88% of the crosses attempted produced nutlets. Nevertheless, incompatibility barriers of varying strength became apparent in  $F_1$  and later generations.
- Although seed set is not an entirely reliable indicator of crossability some general trends were evident. Nutlets were usually obtained after cross-pollination not only between subspecies but also between more distantly related taxa. However, seed set in  $F_1$  and subsequent generations was low and there was evidence of incompatibility at both pre- and post-fertilization levels. Where no nutlets



were formed the pollen tube may never have reached the ovule or, if it did, fertilization may have failed. Where small shrivelled nutlets were produced, which was less common, the endosperm may have degenerated causing death of the embryo, or the embryo may not have developed normally for other reasons.

-- Germination was in general variable and fairly low decreasing considerably in  $F_2$  and in later generations.

-- Pollen stainability was mostly reduced and very variable in combinations between taxa. Mean values were higher in crosses between the subspecies of *S. swainsonii* than in crosses with other species. The pollen stainability was exceptionally high in *candida* x *chrysantha* and *ssp. spreitzenhoferi* x *ssp. virella*.

-- The hybrids usually developed well morphologically. Most characters were intermediate in  $F_1$  and showed continuous variation in  $F_2$  (which according to Grant (1954) would suggest that these characters are determined by multiple factors).

-- Signs of hybrid inviability ranging from lethality to varying degrees of weakness appeared in some combinations.

-- Chromosome pairing was good and meiotic abnormalities were usually few. The reduced pollen fertility in the hybrids is apparently caused by cryptic structural differences in the chromosomes or by genic dissimilarities.

-- Some evidence of relationships became clear. The four subspecies of *S. swainsonii* are closely interrelated. Incompatibility barriers are strongest between *ssp. swainsonii* and *ssp. argolica*; *spruneri* is linked to *S. swainsonii* through *ssp. argolica* and *ssp. melangavica* while *ionica* is more isolated.

In spite of morphological similarity there is evidently genetic disharmony between *candida* and *chrysantha* since hybrid breakdown appeared in later generations.

The two subspecies of *spreitzenhoferi* are closely related and their hybrids were highly fertile.

The other species used in the crossing programme are more distantly related.



## GENERAL CONCLUSIONS

(see also conclusions at the end of MORPHOLOGICAL VARIATION and CROSSING EXPERIMENTS)

The results from the preceding pages show that *S. swainsonii* forms a network of closely related taxa. *Spruneri* has close affinity with *S. swainsonii* but *ionica* is more remote as are the species of the *candida* groups and the other species used in the crossing experiments. All taxa can be crossed but reproductive barriers of varying strength exist and to some extent they reflect the degree of isolation. No gene exchange occurs because the taxa are now geographically separated (except for two points of contact). All taxa have the same chromosome number,  $2n=34$ , and meiotic abnormalities in the hybrids were comparatively few when seen in a broad context.

Microhabitat-correlated variation within populations suggests that selection and recombination have played an important part in the evolution of the species. In general the populations are not small enough for genetic drift to be of importance, but it may have some effect at subpopulation level. Nor can mutation rate be disregarded.

## PALEOGEOGRAPHY OF THE AREA

The paleogeography of the area of distribution may provide a clue to the possible early history of the taxa involved -- but this can only lead to speculation. The paleogeography of the Greek mainland, which is incompletely known, has been sketched by Creutzburg (1963). During the middle Miocene central and southern Greece formed a continuous landmass (Fig. 30 A) which included parts of the present-day Ionian Islands. By the late Pliocene (Fig. 30 B) the landmass had become partly submerged so that it was broken up into several areas. Thus the Isthmus of Corinth, Argolis and Peloponnisos became islands separated from the mainland to the north. Owing to the regression of the sea during the cold periods of the early Pleistocene and to tectonic movements, these areas again became a continuous landmass except for part of the present Gulf of Corinth which was a lake.

The ecological requirements for all taxa are the same -- they grow in rocky calcareous habitats, usually at lower altitudes (though *ssp. swainsonii*, *spruneri* and *candida* ascend to c. 1000 m or more). Since they require this particular type of habitat one cannot merely assume a continuous distribution during times of land connection but must also take topography into consideration. However, the *swainsonii* group (ancestors?) may have had a much wider distribution during the middle Miocene through the early Pliocene when the bodies of water now separating the areas of distribution did not yet exist. In the late Pliocene, as part of the area was submerged the group may have been reduced and split up into smaller isolated entities. Each of these may subsequently have developed along different lines mainly owing to the action of selection, recombination, genetic drift and mutation on the inherent variability. Later, when the sea regressed in the early Pleistocene, some of the areas were again connected and the populations could expand. Part of the



Fig. 30. -- A: Southern Greece during middle Miocene. -- B: Southern Greece during early Pliocene. From Creutzburg 1963.

land, sea, lake.



present Gulf of Corinth was submerged but Attika, the Isthmus of Corinth and Argolis formed a continuous area. In recent times climatic differences between glacial and interglacial periods have undoubtedly caused fluctuations in population size and area with consequent cycles of depletion and replenishment of the gene pool. Thus there have been several opportunities for hybridization and it is very likely that hybrids could become established in natural populations. My experiments suggest that *ssp. melangavica* is of hybrid origin. The present distribution area does not contradict this possibility; it is located at a point where the other taxa could have met in past times. It is impossible to know when hybridization took place but *spruneri*, *ssp. swainsonii* and *ssp. scyronica* were then probably already differentiated. The hybrid populations may subsequently have become isolated and eventually stabilized as the present subspecies. The present-day distribution may be a remnant of a wider range.

While *spruneri* and *ionica* are well-established species and *sspp. swainsonii*, *scyronica* and *melangavica* fairly well-established subspecies, *ssp. argolica* has not yet advanced as far on the road towards speciation. *Ssp. argolica* is more variable than the other subspecies and it is possible that hybridization with *ssp. swainsonii* and *spruneri* has taken place. Or it may be the result of hybridization between these two taxa followed by extensive introgression. The wide morphological variation speaks for more recent hybridization than that in *ssp. melangavica*. Or hybridization may have occurred several times, both with *ssp. swainsonii* in a more distant past, and with *spruneri* more recently; this would be paleogeographically possible.

Now, *ionica* is geographically and genetically somewhat removed from the rest of the group. The present Ionian Islands were part of the mainland during the early Pliocene; in the late Pliocene Leukas, Ithaki and part of Kephallinia were connected with the mainland while Zante was isolated; and during the early Pleistocene Zante, Ithaki and Kephallinia formed one island whereas Leukas

remained part of the mainland. It is not known whether there were then any suitable habitats between the present areas of distribution and if so whether *ionica* had any contact with the rest of the group.

Geographically, *candida* on Mt Taiyetos and *chrysantha* on Mt Parnon have probable been isolated for a considerable time. Mt Taiyetos is a much older formation (from the Triassic period) than Mt Parnon (from the Eocene and Oligocene epochs). Though the two mountains were connected by land during most of the time (separated by lakes during the late Pliocene) the valley between them has probably served as an effective isolation barrier.

Creutzburg's maps show a land-bridge between Kithira and Peloponnisos as late as the early Pleistocene. The few populations of *ssp. spreitzenhoferi* on the Malea Peninsula may be a remnant of a wider distribution from this time. Rechinger (in Greuter and Rechinger 1967) discussed the phytogeography of Kithira and also mentioned other species with a Kithira-Peloponnisos distribution.

With its sole locality at Monemvasia *ssp. virella* is difficult to explain. If *S. spreitzenhoferi* was once distributed over a wider area on the Maléa Peninsula *ssp. virella* may originally have been part of this distribution and later become isolated and differentiated into the present subspecies.

In conclusion, all taxa are presumably relicts of formerly more widespread species complexes. With the fluctuations in climate the populations have presumably fluctuated in size eventually giving rise to the pattern of variation found in the *swainsonii* and *candida* groups today. Recent geological research (Hsü et al. 1973) supports the suggestion that the basic differentiation could well have been established during or strongly influenced by the drastic climatic fluctuations of the Messinian (late Miocene) (cf. Greuter 1979). This pattern of evolutionary history is probably also common in other Mediterranean plant groups of lower altitudes.



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# APPENDIX 1. POPULATIONS USED IN CROSSING EXPERIMENTS.

All populations occupy calcareous habitats.

Plants and seed collected June, July 1973, May, June 1976.

Additional collections by Runemark, Snogerup and Strid.

Populations for which the chromosome number has been determined are marked \*.

## *S. swainsonii* ssp. *swainsonii*

- DPA\* Fokis. Delphi, S cliff face of Mt Parnassos, 600 - 1100 m.
  - DPB\* Fokis. 1 km E of turnoff to Itea along road Delphi - Amfissa, S-facing rocks, 100 m.
  - DPC Fokis. 1 km E of turnoff to Galaxidhion, E-facing cliffs, 920 m.
  - DPD Fokis. 4 km SW of Amfissa, N-facing rocks, 480 m.
  - DPE Fokis. 5 km SSW of Lidhorikion, rocks facing W, 650 m.
  - DPF Fokis. 9 km S of Lidhorikion, small rocks facing W, 710 m.
  - DPC Fokis. 1 km E of turnoff to Galaxidhion, small rocks near road facing SE, 810 m.
  - DPH Fokis. 2 km W of Vourthiera (between Galaxidhion and Amfissa), small rocks facing N and SSW, 720 m.
  - DPI\* Fokis. Along road Delphi - Amfissa at western turnoff to Itea, N-facing rocks, 180 m.
  - DPK Fokis. 2 km E of DPI, W-facing rocks, 140 - 150 m.
  - DPL\* Voiotia. 13 km W of Arakhova, S-facing mountain slope, 590 - 620 m.
  - EJ\* Voiotia. Above Arakhova, S-facing cliff-side, 1500 m.
- S. swainsonii* ssp. *scyronica*
- DPT\* Attiki. 13 km E of Ay. Theodoroi along road to Megara, cliffs facing S, 18 - 60 m.
- S. swainsonii* ssp. *melangavica*
- DL\* Korinthia. Loutraki near church above town, WSW-facing rock, 510 m.
  - LA\* Korinthia. 1 km N of Loutraki, cliffs facing S, 90 m.
  - LB\* Korinthia. 5 km N of Loutraki, cliffs facing NW, 290 m.
  - LC\* Korinthia. 11 km NW of Loutraki, rocks facing S, 190 m.
  - VA Korinthia. Akr. Melangavi 18 km NW of Loutraki, cliffs facing E, 60 m.

## *S. swainsonii* ssp. *argolica*

- DPX\* Korinthia. S Korinthos at Acrokorinth to the right of entrance to the old fort, cliff facing W, 400 m.
  - XC\* Korinthia. Akrokorinth. NNW side of cliff, 260 m.
  - CH\* Korinthia. Akrokorinth, E side of cliff, 340 - 360 m.
  - DPY Argolis. 2 km NNE of Navplion just E of the road to Argos, rocks facing W, 30 m.
  - DPZ\* Argolis. Navplion, W-facing cliff SW of town.
  - AA\* Argolis. 5 km NNE of Dhrepanon (E Navplion), rocks facing E-NE, 150 m.
  - AB\* Argolis. 1 km SW of Leukakia, rocks facing NE, 110 - 120 m.
  - AC\* Argolis. 2 km S of Dhrepanon, rocks facing SE, 70 m.
  - AD\* Argolis. 2.5 - 3 km E of Dhrepanon, coastal rocks facing SE, 20 - 30 m.
  - AE\* Argolis. 5 km E of Dhrepanon, rocks facing WSW, 90 m.
  - AF\* Argolis. 6 - 7 km W of Dhrepanon.
  - AG\* Argolis. 2 km NNW of Kalloni, E-facing cliffs, 100 m.
  - AH\* Argolis. 3 - 7 km SW of Methana, coastal rocks N- and S-facing, 50 - 90 m.
  - EA\* Argolis. 200 m N of junction of road to Nēa Epidhavros and main road to Ligourion, ravine running E-W, 105 m.
  - EB\* Argolis. Idhra, cliffs above town.
  - EC\* Argolis. NW of turnoff to Irion.
- S. spruneri*
- DPP\* Voiotia. 1 km N of turnoff to Aktraifnion (c. 20 km NNW of Thivae), W-facing rocks, 120 m.
  - DPS\* Attiki. Parnis Oros. S- and E-facing slopes, 540 - 990 m.
  - CI\* Attiki. Yerania Ori, S-facing rocks 980 - 1000 m.
  - CK Attiki. 2 km NW of Kaza (S Thivae), W-facing cliffs near the road, 570 m.
- S. ionica*
- CZ Kefallinia. 6 km S of Assos, W-facing rocks 400 m.
  - DA Kefallinia. 2 km S of Assos, stone walls W-facing, 290 m.
  - DB Kefallinia. 0.5 km S of Assos, rocks 50 m. Also Snogerup (TR), June 12, 1966.
  - DC Kefallinia. Peninsula W of Assos, E-facing rock 10 m.
  - DE\* Kefallinia. Poros, ravine running E-W, 10 - 30 m. Also Snogerup (TT), June 15, 1966.
  - DF Kefallinia. 1 km NW of Poros, cliffs facing E, 50 - 100 m.
  - DG\* Ithaki. Near road to Monastery Katharon, rocks in phrygana facing SW, 210 m.
  - DH\* Levkas. Vasiliki, SW-facing cliffs, 20 - 30 m.

- DK\* Levkas. Cape Tsouanna, cliffs facing all directions, 50 - 130 m.
- S. euboica
- R1435 Euboea. 5 km S of Akr. Kafiyeys. Runemark, June 22, 1958.
- S. candida
- AO\* Lakonia. Mistras (W Sparti) near the old castle, small rocks facing E, 500 m.
- AP Lakonia. 5 Mistras, N-facing side of ravine, 380 m.
- AR\* Lakonia. E slopes of Taïetos Oros above Ay. Ioannis (W Sparti), 400 - 550 m. Also Strid (BAR), July 3, 1970.
- AS\* Lakonia. 2 km SW of Tripi (W Sparti) along road to Kalamai, cliffs facing N, 540 m.
- AT Lakonia. Shady ravine W of Xirokambi by old bridge, N- and S-facing, 300 m.
- BS Lakonia. Taïetos Oros. Near Kastania c. 25 km NW of Yithion, N-facing rocks, 450 m.
- CC\* Messinia. 4 - 5 km N of Kardhamili (S Kalamai), W-facing rocks, 280 m.
- CD Messinia. 26 km S of Kalamai, E-facing rocks, 460 m.
- CE Messinia. 18 km S of Kalamai, rocks facing SE, 320 m.
- CF\* Messinia. 13 km S of Kalamai, W- and SW-facing rocks, 300 m.
- CG Messinia. Above Pidhima (10 km S of Tsoukaleika), W-facing rocks, 50 m.
- S. chrysantha
- TC Arkadhia. 2 km SE of Astros, rocks facing NE, 30 m.
- AI Arkadhia. 1 - 3 km from Ay. Petros (c. 25 km SE of Tripolis) along road to Astros, rocks facing S-SW, 890 m.
- AK Arkadhia. C. 4 km NNE of Ay. Nikolaos (20 km. SE of Tripolis), rocks facing W, 1100 m.
- AL\* Lakonia. 19 km N of Sparti, rocks facing E, 780 m.
- AM\* Lakonia. 17 km N of Sparti, in ravine, rocks facing S and N, 740 m.
- AU Lakonia. 1 km W of Coritaa (SE Sparti), E-facing rocks, 350 m.
- AY Lakonia. 5 km W of Geraki, ravine running E-W, 350 m.
- AZ Lakonia. 2 km N of intersection near Geraki along road to Kozmas, 460 m.
- BA Lakonia. Along road Vrontamas - Paniyirista, rocks facing E, 200 m.
- BP\* Lakonia. W Molaoi, cliffs facing N, 250 m.
- BD Lakonia. 2 km N of Molaoi, E-facing rocks, 60 m.
- BC\* Lakonia. Monemvasia Peninsula, N and W side of cliff, 20 - 50 m.
- BL Lakonia. 8 km S of Monemvasia, rocks along road facing S, 190 m.
- BR Lakonia. Near village of Kabor (8 km N of Neapolis), cliffs facing W, 150 m.
- BAD\* Lakonia. 4 km W of Erika, rocks near the road. Also Strid, June 30, 1970.
- BAH\* Lakonia. 3 km N of Ay. Apostoli (N Neapolis), SW-facing rocks, 120 m. Also Strid, July 2, 1970.
- BAK Lakonia. 2 km E of Ellinikon (Neapolis - Monemvasia), W-facing rocks 200 m.
- BAN Lakonia. 4 km from Monemvasia along road to Sikea, N-facing cliffs, 50 m.
- S. spreitzenhoferi ssp. spreitzenhoferi
- BAE\* Kithira. 1 km S of Ay. Pelayia, rocks in ravine, 200 m. Strid, June 30, 1970.
- BAF\* Kithira. 3 km W of Diakofti, ravine running E-W, 100 m. Strid, July 1, 1970.
- BAG Kithira. 3 km NW of Avlemonas, NE-facing cliffs 100 - 150 m. Strid, July 1, 1970.
- BAV\* Lakonia. Neapolis, rocks and stone walls. Strid June 30, 1970.
- BO\* Lakonia. N of Neapolis, rock ledge facing NE, 20 m.
- BP\* Lakonia. N of Neapolis, rocks by small cave facing NE, 20 m.
- S. spreitzenhoferi ssp. virella
- SU\* Lakonia. Monemvasia, large vertical rock, SW side c. 50 m, N side 20 - 50 m, S side 50 - 100 m. Also Strid, July 14, 1970.
- S. messeniaca
- SS\* Lakonia. 8 km SW-WSW of Yithion, rocks facing SW, 40 m. Strid, July 14, 1966.
- BT Lakonia. 1 km N of Yithion, N-facing rocks, 110 m.
- BU Lakonia. 9 - 10 km S of Yithion, rocks facing SE, 50 - 80 m.
- BY\* Lakonia. E-facing coastal cliffs SW of Yerolimn, 40 m.
- BZ\* Lakonia. 2 km N of Aeropolis, coastal rocks facing N, 30 - 50 m.
- CA Lakonia. 2 km N of Aeropolis, W-facing cliffs near the sea, c. 60 m.
- CB\* Messinia. 18 km N of Aeropolis (N of Ay. Nikon) N-facing cliffs in ravine, 450 m.
- S. mollissima
- CL\* Corfu. Mount Deka, 1 - 3 km N of Makrata (12 km S of Kerkira), rocks facing NW, 190 m.
- CN\* Corfu. Mt Pantokrator 2 km WNW of Nissaki, S-facing cliffs N of road, 150 - 200 m. Also Snogerup (TH), June 17, 1966.
- CO\* Corfu. Spartilla, rocks and stone walls facing E, 220 m.
- CP Corfu. 5 km E of Palaokastritsa, rocks facing SW, 100 m.
- CR Corfu. Palaokastritsa, along road to monastery, 50 - 110 m.
- CS Corfu. 2 km S of Benitzes, rocks facing E.
- CT\* Corfu, 1 km N of Benitzes, rocks facing E. Also Snogerup (TH), June 6, 1966.

CU Corfu. 1 km E of Palaokastritsa, S-facing stone walls, 110 m.  
CX\* Corfu. 0.5 - 4 km E of Lakones, S-facing stone walls along road, 110 m.

S. tetragona

DPO\* Euboea. Dirphis Oros, 1 km N of Steni, S-facing cliffs, 660 - 700 m.



## APPENDIX 2. CROSSES

% nutlets = % of maximum production, 4 nutlets per flower.

|                      | No. of<br>comb.<br>betw.<br>pop. | No. of<br>flow.<br>poll. | No. of<br>nutl. | %<br>nutl. |
|----------------------|----------------------------------|--------------------------|-----------------|------------|
| mollissima           | 4                                | 20                       | 28              | 35.0       |
| tetragona            | -                                |                          |                 |            |
| <i>S. spruneri</i>   |                                  |                          |                 |            |
| x                    |                                  |                          |                 |            |
| ssp. swainsonii      | 2                                | 42                       | 76              | 45.2       |
| ssp. scyronica       | 3                                | 40                       | 33              | 20.6       |
| ssp. melangavica     | 1                                | 13                       | 13              | 25.0       |
| ssp. argolica        | 7                                | 42                       | 98              | 58.3       |
| ionica               | 1                                | 4                        | 0               | 0          |
| euboica              | 1                                | 2                        | 0               | 0          |
| candida              | 3                                | 9                        | 10              | 27.8       |
| chrysantha           | 1                                | 1                        | 0               | 0          |
| ssp. spreitzenhoferi | -                                |                          |                 |            |
| ssp. virella         | 1                                | 2                        | 0               | 0          |
| messeniaca           | -                                |                          |                 |            |
| mollissima           | -                                |                          |                 |            |
| tetragona            | 1                                | 2                        | 0               | 0          |
| <i>S. ionica</i>     |                                  |                          |                 |            |
| x                    |                                  |                          |                 |            |
| ssp. swainsonii      | 2                                | 20                       | 48              | 60.0       |
| ssp. scyronica       | 4                                | 25                       | 33              | 33.0       |
| ssp. melangavica     | -                                |                          |                 |            |
| ssp. argolica        | 4                                | 24                       | 25              | 26.0       |
| spruneri             | 1                                | 46                       | 23              | 12.8       |
| euboica              | 1                                | 17                       | 13              | 19.1       |
| candida              | 1                                | 13                       | 40              | 76.9       |
| chrysantha           | 1                                | 33                       | 12              | 9.1        |
| spreitzenhoferi      | 1                                | 24                       | 19              | 19.8       |
| ssp. virella         | 1                                | 10                       | 4               | 10.0       |
| messeniaca           | 2                                | 26                       | 5               | 4.8        |
| mollissima           | 2                                | 28                       | 18              | 16.1       |
| tetragona            | 1                                | 3                        | 9               | 75.0       |
| <i>S. euvoica</i>    |                                  |                          |                 |            |
| x                    |                                  |                          |                 |            |
| ssp. swainsonii      | 7                                | 103                      | 230             | 55.8       |
| ssp. scyronica       | 3                                | 23                       | 17              | 18.5       |
| ssp. melangavica     | 3                                | 12                       | 15              | 31.2       |
| ssp. argolica        | 4                                | 54                       | 100             | 46.3       |
| spruneri             | 4                                | 26                       | 19              | 18.3       |
| ionica               | 2                                | 4                        | 3               | 18.8       |
| euboica              | 1                                | 16                       | 38              | 59.4       |
| chrysantha           | 2                                | 11                       | 16              | 36.4       |
| ssp. spreitzenhoferi | 2                                | 13                       | 16              | 30.8       |
| ssp. virella         | 2                                | 42                       | 21              | 12.6       |
| messeniaca           | 2                                | 9                        | 22              | 61.1       |
| mollissima           | 2                                | 9                        | 19              | 52.8       |
| tetragona            | -                                |                          |                 |            |
| <i>S. chrysantha</i> |                                  |                          |                 |            |
| x                    |                                  |                          |                 |            |
| ssp. swainsonii      | 8                                | 58                       | 56              | 24.1       |
| ssp. scyronica       | 2                                | 25                       | 44              | 44.0       |

|                             | No. of<br>comb.<br>betw.<br>pop. | No. of<br>flow.<br>poll. | No. of<br>nutl. | $\bar{x}$<br>nutl. |
|-----------------------------|----------------------------------|--------------------------|-----------------|--------------------|
| <i>ssp. melangavica</i>     | -                                |                          |                 |                    |
| <i>ssp. argolica</i>        | 7                                | 38                       | 66              | 43.4               |
| <i>spruneri</i>             | 2                                | 34                       | 51              | 37.5               |
| <i>ionica</i>               | 1                                | 2                        | 0               | 0                  |
| <i>euboica</i>              | 1                                | 10                       | 12              | 30.0               |
| <i>candida</i>              | 3                                | 23                       | 22              | 23.9               |
| <i>ssp. spreitzenhoferi</i> | 2                                | 58                       | 16              | 6.9                |
| <i>ssp. virella</i>         | 2                                | 72                       | 40              | 13.9               |
| <i>messeniac</i>            | 1                                | 6                        | 11              | 45.8               |
| <i>mollissima</i>           | 1                                | 7                        | 2               | 7.1                |
| <i>tetragona</i>            | -                                |                          |                 |                    |
| <i>S. messeniaca</i>        |                                  |                          |                 |                    |
| <i>x</i>                    |                                  |                          |                 |                    |
| <i>S. spreitzenhoferi</i>   |                                  |                          |                 |                    |
| <i>ssp. spreitzenhoferi</i> |                                  |                          |                 |                    |
| <i>x</i>                    |                                  |                          |                 |                    |
| <i>ssp. swainsonii</i>      | 4                                | 44                       | 60              | 34.1               |
| <i>ssp. scyronica</i>       | 2                                | 22                       | 54              | 61.4               |
| <i>ssp. melangavica</i>     | 3                                | 22                       | 24              | 27.3               |
| <i>ssp. argolica</i>        | 9                                | 80                       | 98              | 30.6               |
| <i>spruneri</i>             | 2                                | 12                       | 23              | 47.9               |
| <i>ionica</i>               | 3                                | 48                       | 64              | 33.3               |
| <i>euboica</i>              | 1                                | 48                       | 43              | 22.4               |
| <i>candida</i>              | 4                                | 38                       | 35              | 23.0               |
| <i>chrysantha</i>           | 3                                | 45                       | 42              | 23.3               |
| <i>ssp. virella</i>         | 3                                | 83                       | 122             | 36.7               |
| <i>messeniac</i>            | 1                                | 17                       | 22              | 32.4               |
| <i>mollissima</i>           | 2                                | 68                       | 37              | 13.6               |
| <i>tetragona</i>            | 1                                | 6                        | 3               | 17.5               |
| <i>S. spreitzenhoferi</i>   |                                  |                          |                 |                    |
| <i>ssp. virella</i>         |                                  |                          |                 |                    |
| <i>x</i>                    |                                  |                          |                 |                    |
| <i>ssp. swainsonii</i>      | 3                                | 22                       | 17              | 19.3               |
| <i>ssp. scyronica</i>       | 1                                | 12                       | 8               | 16.7               |
| <i>ssp. melangavica</i>     | -                                |                          |                 |                    |
| <i>ssp. argolica</i>        | 2                                | 25                       | 21              | 21.0               |
| <i>ssp. argolica</i>        | 1                                | 2                        | 1               | 12.5               |
| <i>spruneri</i>             |                                  |                          |                 |                    |
| <i>ionica</i>               |                                  |                          |                 |                    |
| <i>euboica</i>              |                                  |                          |                 |                    |
| <i>candida</i>              |                                  |                          |                 |                    |
| <i>chrysantha</i>           |                                  |                          |                 |                    |
| <i>ssp. spreitzenhoferi</i> |                                  |                          |                 |                    |
| <i>ionica</i>               | 1                                | 18                       | 17              | 23.6               |
| <i>euboica</i>              | 1                                | 13                       | 35              | 67.3               |
| <i>candida</i>              | 1                                | 33                       | 22              | 16.7               |
| <i>chrysantha</i>           | 4                                | 29                       | 25              | 21.6               |
| <i>ssp. spreitzenhoferi</i> | 4                                | 24                       | 30              | 31.3               |
| <i>messeniac</i>            | 2                                | 12                       | 5               | 10.4               |
| <i>mollissima</i>           | 2                                | 13                       | 7               | 13.5               |
| <i>tetragona</i>            | -                                |                          |                 |                    |
| <i>S. messeniaca</i>        |                                  |                          |                 |                    |
| <i>x</i>                    |                                  |                          |                 |                    |
| <i>ssp. swainsonii</i>      | 3                                | 39                       | 93              | 59.6               |
| <i>ssp. scyronica</i>       | 2                                | 26                       | 15              | 14.4               |
| <i>ssp. melangavica</i>     | 1                                | 6                        | 4               | 16.7               |
| <i>ssp. argolica</i>        | 2                                | 17                       | 40              | 58.8               |
| <i>spruneri</i>             | 1                                | 52                       | 77              | 37.0               |
| <i>ionica</i>               | 1                                | 20                       | 17              | 21.2               |
| <i>euboica</i>              | 2                                | 9                        | 14              | 38.9               |
| <i>candida</i>              | 2                                | 16                       | 17              | 26.6               |
| <i>chrysantha</i>           | -                                |                          |                 |                    |
| <i>ssp. spreitzenhoferi</i> | 2                                | 42                       | 79              | 47.0               |
| <i>ssp. virella</i>         | 1                                | 5                        | 9               | 45.0               |
| <i>mollissima</i>           | 2                                | 13                       | 18              | 34.6               |
| <i>tetragona</i>            | 1                                | 2                        | 5               | 62.5               |
| <i>S. mollissima</i>        |                                  |                          |                 |                    |
| <i>x</i>                    |                                  |                          |                 |                    |
| <i>ssp. swainsonii</i>      | 3                                | 57                       | 64              | 28.1               |
| <i>ssp. scyronica</i>       | 1                                | 9                        | 21              | 58.3               |
| <i>ssp. melangavica</i>     | 1                                | 9                        | 8               | 22.2               |
| <i>ssp. argolica</i>        | 4                                | 28                       | 44              | 39.3               |
| <i>spruneri</i>             | 3                                | 25                       | 59              | 59.0               |
| <i>ionica</i>               | 1                                | 3                        | 1               | 8.3                |
| <i>euboica</i>              | 1                                | 3                        | 1               | 8.3                |
| <i>candida</i>              | 1                                | 7                        | 5               | 17.9               |
| <i>chrysantha</i>           | 1                                | 3                        | 1               | 8.3                |
| <i>ssp. spreitzenhoferi</i> | 1                                | 7                        | 1               | 3.6                |

|                             | betw. pop. | poll. | F <sub>1</sub> | No. of<br>nutl.<br>seen | Germ.<br>% | No. of<br>plants | No. of<br>flow.<br>poll. | No. of<br>nutl.<br>pro-<br>duced | Σ<br>nutl. | Pollen stain.<br>mean<br>range |
|-----------------------------|------------|-------|----------------|-------------------------|------------|------------------|--------------------------|----------------------------------|------------|--------------------------------|
| <i>S. swainsonii</i>        |            |       |                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. swainsonii</i>      |            |       |                |                         |            |                  |                          |                                  |            |                                |
| x                           |            |       |                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. scyronica</i>       | 2          | 13    | 5              | 63                      | 32         | 16               | 159                      | 112                              | 17.5       | 73 50-98                       |
| <i>ssp. melangavica</i>     | -          |       |                | 32                      | 66         | 17               | 152                      | 70                               | 11.5       | 76 66-94                       |
| <i>ssp. argolica</i>        | -          |       |                | 135                     | 28         | 37               | 470                      | 19                               | 1.0        | 59 30-86                       |
| <i>spruneri</i>             | -          |       |                | 50                      | 18         | 7                | 100                      | 5                                | 1.2        | 42 20-57                       |
| <i>ionica</i>               | 1          | 15    | 2              | 46                      | 33         | 15               | 93                       | 3                                | 0.8        | 57 47-86                       |
| <i>euboica</i>              | 1          | 3     | 3              | 68                      | 32         | 16               | 95                       | 3                                | 0.8        | 43 24-67                       |
| <i>candida</i>              | 1          | 4     | 8              | 61                      | 23         | 11               | 25                       | 5                                | 5.0        | 43(8) 27-65                    |
| <i>chrysantha</i>           | 1          | 5     | 8              | 48                      | 17         | 6                | 28                       | 0                                | 0          | 31(5) 8-46                     |
| <i>ssp. spreitzenhoferi</i> | -          |       |                | 61                      | 43         | 11               | 111                      | 0                                | 0          | 36 4-54                        |
| <i>ssp. virella</i>         | -          |       |                | 93                      | 27         | 12               | 107                      | 1                                | 0.2        | 39 16-82                       |
| <i>messeniac</i>            | -          |       |                | 59                      | 31         | 11               | 177                      | 5                                | 0.7        | 33 17-52                       |
| <i>mollissima</i>           | -          |       |                | 0                       |            |                  |                          |                                  |            |                                |
| <i>tetragona</i>            | -          |       |                | 10                      | 70         | 5                | 74                       | 0                                | 0          | 49 35-54                       |
| <i>S. swainsonii</i>        |            |       |                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. scyronica</i>       |            |       |                |                         |            |                  |                          |                                  |            |                                |
| x                           |            |       |                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. swainsonii</i>      |            |       |                | 47                      | 66         | 22               | 174                      | 24                               | 3.4        | 82 64-97                       |
| <i>ssp. melangavica</i>     |            |       |                | 12                      | 58         | 7                | 89                       | 28                               | 7.9        | 72 6-87                        |
| <i>ssp. argolica</i>        |            |       |                | 9                       | 56         | 4                | 42                       | 5                                | 3.0        | 77 73-82                       |
| <i>spruneri</i>             |            |       |                | 12                      | 42         | 4                | 46                       | 4                                | 2.2        | 42(3) 35-56                    |
| <i>ionica</i>               |            |       |                | 25                      | 56         | 12               | 115                      | 14                               | 3.0        | 52 38-69                       |
| <i>euboica</i>              |            |       |                | 16                      | 56         | 6                | 96                       | 14                               | 3.6        | 50 32-62                       |
| <i>candida</i>              |            |       |                | 18                      | 33         | 5                | 81                       | 1                                | 0.3        | 44 38-59                       |
| <i>chrysantha</i>           |            |       |                | 9                       | 44         | 3                | 37                       | 0                                | 0          | 44 31-51                       |
| <i>ssp. spreitzenhoferi</i> |            |       |                | 23                      | 52         | 11               | 102                      | 0                                | 0          | 29 9-42                        |
| <i>ssp. virella</i>         |            |       |                | 6                       | 50         | 3                | 35                       | 0                                | 0          | 35 26-52                       |
| <i>S. swainsonii</i>        |            |       |                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. melangavica</i>     |            |       |                |                         |            |                  |                          |                                  |            |                                |
| x                           |            |       |                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. swainsonii</i>      |            |       |                | 24                      | 33         | 8                | 86                       | 8                                | 2.5        | 73 59-88                       |
| <i>ssp. scyronica</i>       |            |       |                | 34                      | 38         | 12               | 118                      | 17                               | 3.6        | 84 67-97                       |

The parenthesis indicates the number of plants in which pollen stainability was determined (in combinations where not all plants were tested).

The parenthesis indicates the number of plants in which pollen stainability was determined (in combinations where not all plants were tested).



| F <sub>1</sub>             | No. of<br>nutl.<br>sown | Germ.<br>% | No. of<br>plants | No. of<br>flow.<br>poll. | No. of<br>nutl.<br>pro-<br>duced | %<br>nutl. | Pollen stain.<br>mean<br>range | No. of<br>nutl.<br>sown | Germ.<br>% | No. of<br>plants | No. of<br>flow.<br>poll. | No. of<br>nutl.<br>pro-<br>duced | %<br>nutl. | Pollen stain.<br>mean<br>range |
|----------------------------|-------------------------|------------|------------------|--------------------------|----------------------------------|------------|--------------------------------|-------------------------|------------|------------------|--------------------------|----------------------------------|------------|--------------------------------|
| <i>ssp. argolica</i>       | 20                      | 35         | 5                | 51                       | 20                               | 9.8        | 83                             | 13                      | 62         | 8                | 99                       | 7                                | 1.8        | 30-85                          |
| <i>spruneri</i>            | 14                      | 93         | 13               | 169                      | 13                               | 1.9        | 56                             | 13                      | 77         | 10               | 35                       | 4                                | 2.9        | 45-80                          |
| <i>ionica</i>              | 11                      | 0          |                  |                          |                                  |            |                                | 12                      | 42         | 5                | 49                       | 11                               | 5.6        | 33-75                          |
| <i>euboica</i>             | 4                       | 75         | 3                | 13                       | 0                                | 0          | 41                             | 19                      | 32         | 6                | 153                      | 0                                | 0          | 29-66                          |
| <i>candida</i>             | 3                       | 0          |                  |                          |                                  |            |                                | 4                       | 75         | 3                | 32                       | 5                                | 3.9        | 44-75                          |
| <i>chrysantha</i>          | 6                       | 0          |                  |                          |                                  |            |                                | 5                       | 80         | 4                | 23                       | 14                               | 15.2       | 49-89                          |
| <i>sp. spreitzenhoferi</i> | 2                       | 50         | 1                | 2                        | 1                                | 12.5       | 54                             | 18                      | 22         | 4                | 78                       | 29                               | 9.3        | 28-99                          |
| <i>mollissima</i>          |                         |            |                  |                          |                                  |            |                                | 9                       | 78         | 6                | 66                       | 47                               | 17.8       | 52-65                          |
| <i>ssp. swainsonii</i>     |                         |            |                  |                          |                                  |            |                                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. argolica</i>       |                         |            |                  |                          |                                  |            |                                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. swainsonii</i>     | 77                      | 55         | 32               | 406                      | 98                               | 6.0        | 68                             | 40                      | 76         | 27               | 218                      | 12                               | 1.4        | 37-71                          |
| <i>ssp. scyronica</i>      | 58                      | 59         | 24               | 315                      | 3                                | 0.2        | 76                             | 21                      | 57         | 12               | 104                      | 28                               | 6.7        | 32-99                          |
| <i>ssp. melangavica</i>    | 80                      | 64         | 25               | 516                      | 74                               | 3.6        | 65                             | 7                       | 43         | 2                | 36                       | 1                                | 0.7        | 49-54                          |
| <i>spruneri</i>            | 84                      | 54         | 22               | 297                      | 92                               | 7.7        | 69                             | 37                      | 49         | 18               | 202                      | 11                               | 1.4        | 24-75                          |
| <i>ionica</i>              | 38                      | 55         | 14               | 325                      | 50                               | 3.8        | 48                             | 12                      | 75         | 9                | 74                       | 4                                | 1.4        | 54-88                          |
| <i>euboica</i>             | 27                      | 67         | 12               | 263                      | 3                                | 0.3        | 44                             | 20                      | 80         | 15               | 60                       | 9                                | 3.8        | 46-84                          |
| <i>candida</i>             | 39                      | 15         | 5                | 70                       | 2                                | 0.7        | 43                             | 5                       | 60         | 1                | 7                        | 4                                | 14.3       | -                              |
| <i>chrysantha</i>          | 12                      | 67         | 7                | 137                      | 1                                | 0.2        | 28                             | 3                       | 67         | 2                | 8                        | 2                                | 6.2        | 82-84                          |
| <i>sp. spreitzenhoferi</i> | 21                      | 33         | 4                | 80                       | 32                               | 10.0       | 50                             | 17                      | 65         | 10               | 48                       | 3                                | 1.6        | 22-93                          |
| <i>sp. virella</i>         | 40                      | 90         | 24               | 194                      | 10                               | 1.3        | 46                             | 6                       | 100        | 6                | 4                        | 3                                | 18.8       | 42-97                          |
| <i>messeniaca</i>          | 38                      | 42         | 12               | 118                      | 15                               | 3.2        | 18                             |                         |            |                  |                          |                                  |            |                                |
| <i>mollissima</i>          | 16                      | 63         | 5                | 116                      | 0                                | 0          | 61                             |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. swainsonii</i>     |                         |            |                  |                          |                                  |            |                                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. scyronica</i>      |                         |            |                  |                          |                                  |            |                                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. melangavica</i>    |                         |            |                  |                          |                                  |            |                                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. argolica</i>       |                         |            |                  |                          |                                  |            |                                |                         |            |                  |                          |                                  |            |                                |
| <i>candida</i>             |                         |            |                  |                          |                                  |            |                                |                         |            |                  |                          |                                  |            |                                |
| <i>ionica</i>              |                         |            |                  |                          |                                  |            |                                |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. swainsonii</i>     | 76                      | 24         | 18               | 218                      | 47                               | 5.4        | 43                             | 65                      | 38         | 21               | 369                      | 80                               | 5.4        | 19-65                          |
| <i>ssp. scyronica</i>      | 17                      | 29         | 26               | 512                      | 107                              | 5.2        | 55                             | 17                      | 41         | 5                | 87                       | 6                                | 1.7        | 39-65                          |
| <i>ssp. melangavica</i>    | 13                      | 77         | 10               | 98                       | 12                               | 3.1        | 65                             | 15                      | 80         | 8                | 139                      | 26                               | 4.7        | 23-53                          |
| <i>ssp. argolica</i>       | 96                      | 34         | 22               | 259                      | 101                              | 9.7        | 86                             | 76                      | 30         | 23               | 419                      | 38                               | 2.3        | 29-72                          |
| <i>candida</i>             | 10                      | 20         | 2                | 32                       | 0                                | 0          | 61                             | 18                      | 22         | 3                | 24                       | 1                                | 1.0        | 21-51                          |
| <i>ionica</i>              |                         |            |                  |                          |                                  |            |                                | 3                       | 33         | 1                | 14                       | 4                                | 7.1        | -                              |
| <i>ssp. swainsonii</i>     |                         |            |                  |                          |                                  |            |                                | 38                      | 82         | 26               | 77                       | 38                               | 12.3       | 39-92                          |
| <i>ssp. scyronica</i>      |                         |            |                  |                          |                                  |            |                                | 16                      | 63         | 10               | 80                       | 110                              | 34.4       | 92-99                          |
| <i>ssp. melangavica</i>    |                         |            |                  |                          |                                  |            |                                | 16                      | 44         | 7                | 334                      | 78                               | 5.8        | 63-92                          |
| <i>ssp. argolica</i>       |                         |            |                  |                          |                                  |            |                                | 21                      | 33         | 7                | 36                       | 4                                | 2.8        | 29-89                          |
| <i>candida</i>             |                         |            |                  |                          |                                  |            |                                | 22                      | 73         | 14               | 61                       | 16                               | 6.6        | 71-83                          |
| <i>ionica</i>              |                         |            |                  |                          |                                  |            |                                | 19                      | 95         | 18               | 65                       | 4                                | 1.5        | 28-82                          |
| <i>ssp. swainsonii</i>     | 44                      | 48         | 14               | 178                      | 24                               | 3.4        | 49                             |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. scyronica</i>      | 29                      | 31         | 7                | 144                      | 4                                | 0.7        | 49                             |                         |            |                  |                          |                                  |            |                                |
| <i>ssp. argolica</i>       | 20                      | 70         | 12               | 75                       | 1                                | 0.3        | 59                             |                         |            |                  |                          |                                  |            |                                |
| <i>spruneri</i>            | 23                      | 48         | 11               | 158                      | 17                               | 2.7        | 65                             |                         |            |                  |                          |                                  |            |                                |

| I                         | F <sub>1</sub>       | No. of<br>nutl.<br>sown | germ.<br>% | No. of<br>plants | No. of<br>flow.<br>poll. | No. of<br>nutl.<br>pro-<br>duced | Pollen stain-<br>ing<br>range | mean   | Pollen stain-<br>ing<br>range |
|---------------------------|----------------------|-------------------------|------------|------------------|--------------------------|----------------------------------|-------------------------------|--------|-------------------------------|
| <b>S. chrysanthia</b>     |                      |                         |            |                  |                          |                                  |                               |        |                               |
| x                         | spruneri             | 1                       | 0          |                  |                          |                                  |                               |        |                               |
|                           | ionica               | 17                      | 53         | 9                | 13                       | 1                                |                               | 51     | 30-77                         |
|                           | eubonica             | 25                      | 34         | 20               | 265                      | 23                               |                               | 47     | 21-80                         |
|                           | candida              | 22                      | 59         | 13               | 266                      | 41                               |                               | 63     | 42-72                         |
|                           | chrysanthia          | 25                      | 20         | 5                | 32                       | 20                               |                               | 77     | 57-90                         |
|                           | ssp. spreitzenhoferi | 33                      | 60         | 18               | 454                      | 255                              |                               | 95     | 71-99                         |
|                           | messeniacia          | 5                       | 40         | 4                | 40                       | 8                                |                               | 59     | 46-79                         |
|                           | mollissima           | 7                       | 29         | 2                | 35                       | 1                                |                               | 75     | 70,79                         |
|                           | S. messeniaca        |                         |            |                  |                          |                                  |                               |        |                               |
| x                         | ssp. swainsonii      | 63                      | 80         | 11               | 93                       | 9                                |                               | 39(10) | 28-49                         |
|                           | ssp. scyronica       | 12                      | 83         | 2                | 159                      | 9                                |                               | 37     | 22-22                         |
|                           | ssp. melangavica     | 3                       | 67         | 1                | 24                       | 0                                |                               | 37     | -                             |
|                           | ssp. argolica        | 20                      | 20         | 6                | 32                       | 2                                |                               | 23     | 17-28                         |
|                           | spruneri             | 28                      | 71         | 16               | 218                      | 14                               |                               | 42     | 30-54                         |
|                           | ionica               | 11                      | 65         | 11               | 32                       | 11                               |                               | 70     | 62-79                         |
|                           | eubonica             | 14                      | 86         | 11               | 113                      | 5                                |                               | 59     | 31-82                         |
|                           | candida              | 17                      | 65         | 11               | 48                       | 12                               |                               | 63     | 39-94                         |
|                           | ssp. spreitzenhoferi | 18                      | 50         | 3                | 152                      | 17                               |                               | 51     | 38-78                         |
|                           | ssp. virella         | 9                       | 44         | 4                | 26                       | 4                                |                               | 32     | 16-30                         |
|                           | mollissima           | 16                      | 69         | 10               | 165                      | 28                               |                               | 44     | 31-67                         |
|                           | tetrastigma          | 5                       | 60         | 3                | 52                       | 43                               |                               | 91     | 65-92                         |
|                           | S. mollissima        |                         |            |                  |                          |                                  |                               |        |                               |
| x                         | ssp. swainsonii      | 64                      | 75         | 16               | 232                      | 5                                |                               | 48     | 16-58                         |
|                           | ssp. scyronica       | 13                      | 0          |                  |                          |                                  |                               |        |                               |
|                           | ssp. melangavica     | 8                       | 13         | 1                | 16                       | 0                                |                               | 63     | -                             |
|                           | ssp. argolica        | 44                      | 64         | 15               | 278                      | 20                               |                               | 72     | 45-89                         |
|                           | spruneri             | 53                      | 60         | 17               | 142                      | 10                               |                               | 72     | 34-76                         |
|                           | ionica               | 1                       | 0          |                  |                          |                                  |                               |        |                               |
|                           | eubonica             | 1                       | 100        | 1                | 6                        | 0                                |                               | 46     | -                             |
|                           | candida              | 5                       | 0          |                  |                          |                                  |                               |        |                               |
|                           | chrysanthia          | 1                       | 0          |                  |                          |                                  |                               |        |                               |
|                           | ssp. spreitzenhoferi | 1                       | 0          |                  |                          |                                  |                               |        |                               |
|                           | ssp. virella         | 2                       | 100        | 2                | 14                       | 0                                |                               | 48     | 46,50                         |
|                           | tetrastigma          | 3                       | 33         | 1                | 5                        | 0                                |                               | 88     | -                             |
| <b>S. spreitzenhoferi</b> |                      |                         |            |                  |                          |                                  |                               |        |                               |
| ssp. virella              |                      |                         |            |                  |                          |                                  |                               |        |                               |
| x                         | ssp. swainsonii      | 16                      | 56         | 6                | 61                       | 1                                |                               | 31     | 23-43                         |
|                           | ssp. scyronica       | 3                       | 43         | 3                | 66                       | 1                                |                               | 30     | 29-31                         |
|                           | ssp. argolica        | 12                      | 83         | 8                | 142                      | 0                                |                               | 57     | 46-66                         |

| 1                    | No. of<br>nutl.<br>sown | Germ.<br>Z | No. of<br>plants | No. of<br>flow.<br>poll. | No. of<br>nutl.<br>pro-<br>duced | Z<br>nutl. | Pollen<br>mean<br>range | 9     | No. of<br>nutl.<br>sown | Germ.<br>Z | No. of<br>plants | No. of<br>flow.<br>poll. | No. of<br>nutl.<br>pro-<br>duced | Z<br>nutl. | Pollen<br>mean<br>range |
|----------------------|-------------------------|------------|------------------|--------------------------|----------------------------------|------------|-------------------------|-------|-------------------------|------------|------------------|--------------------------|----------------------------------|------------|-------------------------|
| S. swainsonii        |                         |            |                  |                          |                                  |            |                         |       |                         |            |                  |                          |                                  |            |                         |
| asp. melangavica     | 5                       | 100        | 5                | 58                       | 0                                | 0          | 53                      | 45-68 |                         |            |                  |                          |                                  |            |                         |
| x                    | 2                       | 100        | 2                | 32                       | 7                                | 5.5        | 63                      | 61,65 | 7                       | 14         | 1                | 9                        | 2                                | 5.6        | 95                      |
| ssp. swainsonii      | 3                       | 100        | 3                | 26                       | 3                                | 2.9        | 70                      | 62-80 | 16                      | 50         | 6                | 64                       | 7                                | 2.7        | 68                      |
| ssp. scyronica       | 8                       | 88         | 7                | 15                       | 1                                | 1.7        | 65                      | 56-79 | 13                      | 69         | 9                | 103                      | 35                               | 8.5        | 73                      |
| ssp. argolica        | 8                       | 88         | 6                | 30                       | 3                                | 2.5        | 90                      | 79-95 | 10                      | 50         | 5                | 5                        | 2                                | 10.0       | 62                      |
| sprunerii            |                         |            |                  |                          |                                  |            |                         |       | 1                       | 0          |                  |                          |                                  |            |                         |
| mollissima           |                         |            |                  |                          |                                  |            |                         |       |                         |            |                  |                          |                                  |            |                         |
| S. swainsonii        |                         |            |                  |                          |                                  |            |                         |       |                         |            |                  |                          |                                  |            |                         |
| ssp. argolica        |                         |            |                  |                          |                                  |            |                         |       |                         |            |                  |                          |                                  |            |                         |
| x                    |                         |            |                  |                          |                                  |            |                         |       |                         |            |                  |                          |                                  |            |                         |
| ssp. swainsonii      | 48                      | 23         | 8                | 95                       | 74                               | 19.5       | 58                      | 4-95  | 65                      | 32         | 15               | 128                      | 48                               | 9.4        | 73                      |
| ssp. scyronica       | 54                      | 56         | 29               | 249                      | 111                              | 11.1       | 82                      | 1-99  | 3                       | 100        | 3                | 32                       | 2                                | 1.6        | 87                      |
| ssp. melangavica     | 15                      | 67         | 10               | 168                      | 5                                | 0.7        | 53                      | 0-97  | 58                      | 47         | 25               | 274                      | 32                               | 2.9        | 57                      |
| ssp. argolica        | 5                       | 20         | 1                |                          |                                  |            |                         |       | 64                      | 41         | 24               | 200                      | 55                               | 6.9        | 56                      |
| sprunerii            | 3                       | 33         | 1                | 5                        | 2                                | 10.0       | 56                      | -     | 37                      | 41         | 11               | 80                       | 20                               | 6.2        | 42                      |
| ionica               | 3                       | 100        | 2                | 9                        | 0                                | 0          | 52                      | 51,52 | 3                       | 100        | 2                | 28                       | 5                                | 4.5        | 21                      |
| ssp. melangavica     | 5                       | 40         | 2                | 16                       | 5                                | 7.8        | 53                      | 40,66 | 2                       | 50         | 1                | 41                       | 0                                | 0          | 65                      |
| ssp. argolica        | 1                       | 0          |                  |                          |                                  |            |                         |       | 1                       | 0          |                  |                          |                                  |            | -                       |
| chrysantha           |                         |            |                  |                          |                                  |            |                         |       |                         |            |                  |                          |                                  |            |                         |
| ssp. spreitzenhoferi |                         |            |                  |                          |                                  |            |                         |       | 21                      | 67         | 14               | 207                      | 111                              | 13.4       | 57(13)                  |
| ssp. virella         |                         |            |                  |                          |                                  |            |                         |       | 10                      | 40         | 3                | 68                       | 3                                | 1.1        | 59                      |
| messeniaca           |                         |            |                  |                          |                                  |            |                         |       | 9                       | 89         | 7                | 96                       | 10                               | 2.6        | 37                      |
|                      |                         |            |                  |                          |                                  |            |                         |       |                         |            |                  |                          |                                  |            |                         |
| S. sprunerii         |                         |            |                  |                          |                                  |            |                         |       |                         |            |                  |                          |                                  |            |                         |
| x                    | 5                       | 40         | 2                | 21                       | 6                                | 7.1        | 58                      | 54,63 |                         |            |                  |                          |                                  |            |                         |
| ssp. swainsonii      |                         |            |                  |                          |                                  |            |                         |       | 44                      | 18         | 8                | 68                       | 20                               | 7.4        | 57                      |
| ssp. scyronica       |                         |            |                  |                          |                                  |            |                         |       | 62                      | 42         | 17               | 161                      | 31                               | 4.8        | 64                      |
| ssp. melangavica     |                         |            |                  |                          |                                  |            |                         |       | 10                      | 60         | 6                | 97                       | 33                               | 8.5        | 75                      |
| ssp. argolica        | 20                      | 40         | 6                | 65                       | 5                                | 1.9        | 75(5)                   | 36-99 | 66                      | 47         | 25               | 214                      | 101                              | 11.8       | 88(22)                  |
| melangavica          | 28                      | 54         | 13               | 177                      | 16                               | 2.3        | 68                      | 9-97  |                         |            |                  |                          |                                  |            | 46-98                   |
| argolica             | 5                       | 40         | 1                | 10                       | 0                                | 0          | 81                      | -     |                         |            |                  |                          |                                  |            |                         |
| eri                  | 4                       | 0          |                  |                          |                                  |            |                         |       |                         |            |                  |                          |                                  |            |                         |
| swainsonii           | 14                      | 50         | 5                | 50                       | 9                                | 4.5        | 56                      | 20-78 | 16                      | 31         | 5                | 28                       | 10                               | 8.9        | 53                      |
| melangavica          | 11                      | 27         | 3                | 34                       | 4                                | 2.9        | 79                      | 66-86 | 4                       | 50         | 2                | 26                       | 4                                | 3.8        | 83                      |
| argolica             | 1                       | 0          |                  |                          |                                  |            |                         |       | 1                       | 0          |                  |                          |                                  |            |                         |
| sprunerii            |                         |            |                  |                          |                                  |            |                         |       | 17                      | 12         | 2                | 0                        |                                  |            |                         |



| F <sub>2</sub>       | No. of<br>nutl.<br>sown | Germ.<br>% | No. of<br>plants | No. of<br>flow.<br>poll. | No. of<br>nutl.<br>pro-<br>duced | %<br>nutl. | Pollen stain. %<br>mean range | F <sub>2</sub>       | No. of<br>nutl.<br>sown | Germ.<br>% | No. of<br>plants | No. of<br>flow.<br>poll. | No. of<br>nutl.<br>pro-<br>duced | %<br>nutl. | Pollen stain. %<br>mean range |
|----------------------|-------------------------|------------|------------------|--------------------------|----------------------------------|------------|-------------------------------|----------------------|-------------------------|------------|------------------|--------------------------|----------------------------------|------------|-------------------------------|
| euboica              | 7                       | 29         | 2                | 10                       | 0                                | 0          | 44                            | S. chrysantha        |                         |            |                  |                          |                                  |            |                               |
| candida              | 4                       | 50         | 2                | 18                       | 0                                | 0          | 68(1)                         | x                    |                         |            |                  |                          |                                  |            |                               |
| chrysantha           | 11                      | 9          | 1                | 5                        | 12                               | 60.0       | 66                            | ssp. swainsonii      | 2                       | 50         | 1                | -                        | 29                               | -          | -                             |
| ssp. spreitzenhoferi | 0                       |            |                  |                          |                                  |            |                               | ssp. argolica        | 3                       | 0          |                  |                          |                                  |            |                               |
| ssp. virella         | 5                       | 0          |                  |                          |                                  |            |                               | spruneri             | 2                       | 50         | 1                | -                        |                                  |            |                               |
| messeniacia          | 14                      | 64         | 8                | 22                       | 6                                | 6.8        | 72                            | euboica              | 1                       | 0          |                  |                          |                                  |            |                               |
| mollissima           | 29                      | 59         | 17               | 73                       | 22                               | 7.5        | 72                            | candida              | 32                      | 53         | 6                | 38                       | 31                               | 20.4       | 85 56-97                      |
| tetragona            | 39                      | 41         | 12               | 94                       | 56                               | 14.9       | 68                            | ssp. spreitzenhoferi | 2                       | 0          |                  |                          |                                  |            |                               |
| S. euboica           |                         |            |                  |                          |                                  |            |                               | ssp. virella         | 25                      | 16         | 4                | 32                       | 0                                | 0          | 78 63-94                      |
| x                    |                         |            |                  |                          |                                  |            |                               | messeniacia          | 2                       | 0          |                  |                          |                                  |            |                               |
| ssp. swainsonii      | 12                      | 33         | 4                | 42                       | 0                                | 0          | 71                            | S. spreitzenhoferi   |                         |            |                  |                          |                                  |            |                               |
| ssp. scyronica       | 20                      | 50         | 8                | 113                      | 3                                | 0.7        | 82                            | ssp. spreitzenhoferi |                         |            |                  |                          |                                  |            |                               |
| ssp. melangavica     | 1                       | 0          |                  |                          |                                  |            |                               | x                    |                         |            |                  |                          |                                  |            |                               |
| ssp. argolica        | 11                      | 36         | 4                | 45                       | 4                                | 2.2        | 68                            | ssp. swainsonii      | 1                       | 0          |                  |                          |                                  |            |                               |
| spruneri             | 4                       | 100        | 4                | 19                       | 0                                | 0          | 56                            | ssp. scyronica       | 20                      | 55         | 9                | 165                      | 173                              | 26.2       | 75 26-95                      |
| ionica               | 9                       | 33         | 5                | 46                       | 1                                | 0.5        | 53                            | ssp. melangavica     | 0                       |            |                  |                          |                                  |            |                               |
| candida              | 4                       | 50         | 2                | 0                        | 0                                |            | 59                            | ssp. argolica        | 22                      | 45         | 7                | 107                      | 19                               | 4.4        | 48 36-87                      |
| chrysantha           | 2                       | 100        | 1                | -                        | -                                |            |                               | spruneri             | 15                      | 40         | 6                | 63                       | 24                               | 9.5        | 77 54-92                      |
| ssp. virella         | 3                       | 33         | 1                | 51                       | 0                                | 0          | 48                            | ionica               | 35                      | 29         | 10               | 45                       | 10                               | 5.6        | 67 55-85                      |
| tetragona            | 3                       | 100        | 3                | 7                        | 0                                | 0          | 53                            | euboica              | 0                       |            |                  |                          |                                  |            |                               |
| S. candida           |                         |            |                  |                          |                                  |            |                               | candida              | 118                     | 10         | 12               | 19                       | 11                               | 14.5       | 67 53-96                      |
| x                    |                         |            |                  |                          |                                  |            |                               | chrysantha           | 95                      | 36         | 25               | 59                       | 14                               | 5.9        | 72 36-95                      |
| ssp. swainsonii      | 46                      | 37         | 13               | 159                      | 73                               | 11.5       | 61                            | ssp. virella         | 49                      | 18         | 9                | 42                       | 25                               | 14.9       | 84 60-99                      |
| ssp. scyronica       | 6                       | 67         | 3                | 43                       | 4                                | 2.3        | 65                            | messeniacia          | 18                      | 33         | 6                | 58                       | 14                               | 6.0        | 66 56-71                      |
| ssp. melangavica     | 20                      | 10         | 2                | 2                        | 0                                | 0          | 83                            | mollissima           | 4                       | 25         | 1                | -                        |                                  |            |                               |
| ssp. argolica        | 32                      | 47         | 8                | 35                       | 16                               | 11.4       | 44                            | S. spreitzenhoferi   |                         |            |                  |                          |                                  |            |                               |
| spruneri             | 1                       | 100        | 1                | 14                       | 0                                | 0          | 53                            | ssp. virella         |                         |            |                  |                          |                                  |            |                               |
| ionica               | 4                       | 0          |                  |                          |                                  |            |                               | x                    |                         |            |                  |                          |                                  |            |                               |
| euboica              | 21                      | 57         | 10               | 11                       | 0                                | 0          | 53                            | ssp. swainsonii      | 1                       | 100        | 1                | 32                       | 12                               | 9.4        | 51 -                          |
| chrysantha           | 81                      | 16         | 13               | 21                       | 18                               | 21.4       | 81                            | ssp. scyronica       | 1                       | 0          |                  |                          |                                  |            |                               |
| ssp. spreitzenhoferi | 58                      | 3          | 2                | 9                        | 0                                | 0          | 45                            | ionica               | 1                       | 0          |                  |                          |                                  |            |                               |
| ssp. virella         | 4                       | 25         | 1                | -                        | -                                |            |                               | euboica              | 21                      | 24         | 4                | 73                       | 13                               | 4.5        | 74 59-87                      |
| messeniacia          | 16                      | 94         | 9                | 22                       | 3                                | 3.4        | 74                            | candida              | 41                      | 15         | 5                | 6                        | 0                                | 0          | 56 43-68                      |
| mollissima           | 4                       | 25         | 0                |                          |                                  |            |                               | chrysantha           | 12                      | 50         | 5                | 75                       | 5                                | 1.7        | 72 42-96                      |
|                      |                         |            |                  |                          |                                  |            |                               | ssp. spreitzenhoferi | 234                     | 35         | 35               | 26                       | 27                               | 26.0       | 90 67-99                      |



| $F_2$                | No. of<br>nutl.<br>sown | Germ.<br>% | No. of<br>plants | No. of<br>flow.<br>poll. | No. of<br>nutl.<br>pro-<br>duced | Z<br>nutl. | Pollen stain, %<br>mean<br>range |
|----------------------|-------------------------|------------|------------------|--------------------------|----------------------------------|------------|----------------------------------|
| messeniac            | 8                       | 63         | 5                | 24                       | 1                                | 1.0        | 73 68-77                         |
| mollissima           | 1                       | 0          |                  |                          |                                  |            |                                  |
| S. messeniaca        |                         |            |                  |                          |                                  |            |                                  |
| x                    |                         |            |                  |                          |                                  |            |                                  |
| ssp. swainsonii      | 7                       | 0          |                  |                          |                                  |            |                                  |
| ssp. scytronica      | 9                       | 78         | 6                | 9                        | 0                                | 0          | 55(4) 0-79                       |
| ssp. melangavica     | 0                       |            |                  |                          |                                  |            |                                  |
| spruneri             | 16                      | 44         | 6                | 71                       | 22                               | 7.7        | 53 34-80                         |
| ionica               | 15                      | 47         | 7                | 6                        | 6                                | 25.0       | 73 66-77                         |
| euboica              | 4                       | 0          |                  |                          |                                  |            |                                  |
| candida              | 12                      | 58         | 4                | 24                       | 0                                | 0          | 59 25-78                         |
| ssp. spreitzenhoferi | 24                      | 25         | 4                | 37                       | 14                               | 9.5        | 43 21-86                         |
| ssp. virella         | 4                       | 25         | 1                | 6                        | 3                                | 12.5       | 56 -                             |
| mollissima           | 20                      | 30         | 4                | -                        | -                                | -          | -                                |
| tetragona            | 24                      | 70         | 17               | 60                       | 55                               | 22.9       | 74 33-96                         |
| S. mollissima        |                         |            |                  |                          |                                  |            |                                  |
| x                    |                         |            |                  |                          |                                  |            |                                  |
| ssp. swainsonii      | 5                       | 0          |                  |                          |                                  |            |                                  |
| ssp. argolica        | 17                      | 41         | 5                | 25                       | 5                                | 5.0        | 53 8-88                          |
| spruneri             | 9                       | 11         | 1                | -                        | -                                | -          | 61 -                             |
| . tetragona          |                         |            |                  |                          |                                  |            |                                  |
| ssp. swainsonii      | 0                       |            |                  |                          |                                  |            |                                  |
| ssp. argolica        | 7                       | 100        | 5                | 26                       | 5                                | 4.8        | 56 20-77                         |
| spruneri             | 3                       | 0          |                  |                          |                                  |            |                                  |
| ionica               | 1                       | 100        | 1                | -                        | -                                | -          | 90 -                             |
| euboica              | 3                       | 100        | 3                | 23                       | 0                                | 0          | 80 70-96                         |





